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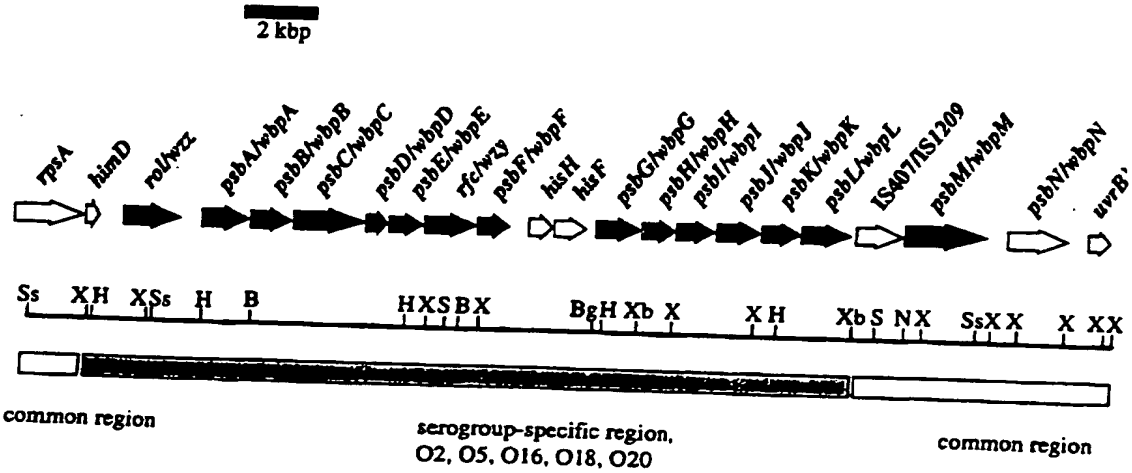

The *Pseudomonas aeruginosa* O5 *wbp* gene cluster and flanking DNA

(57) Abstract

Nucleic acid molecules encoding proteins involved in the synthesis and assembly of O-antigen in *P. aeruginosa*; and proteins encoded by the nucleic acid molecules are described. Methods are disclosed for detecting *P. aeruginosa* in a sample by determining the presence f the proteins or a nucleic acid m lecule encoding the proteins in the sample.

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PROTEINS INVOLVED IN THE SYNTHESIS AND ASSEMBLY OF O-ANTIGEN IN *PSEUDOMONAS AERUGINOSA*

FIELD OF THE INVENTION

The invention relates to novel nucleic acid molecules encoding prot ins involved in the synthesis and assembly of O-antigen in *P. aeruginosa;* the novel proteins encoded by the nucleic acid molecules; and, uses of the proteins and nucleic acid molecules.

BACKGROUND OF THE INVENTION

The opportunistic pathogen *P. aeruginosa* remains a problem in the nosicomial infection of immunocompromised individuals. *P. aeruginosa* infections are particularly a problem in burn patients, people receiving medical implants, and in individuals suffering from cystic fibrosis (Fick, R.B. Jr., 1993). The organism is intrinsically resistant to many antibiotics and capable of forming biofilms which are recalcitrant to treatment. Several virulence factors have been identified in the pathogenesis of *P. aeruginosa* infections, including proteins such as exotoxin A, proteases, and exopolysaccharides including alginate and lipopolysaccharide (LPS). The LPS of *P. aeruginosa* is typical of Gram-negative bacteria, composed of lipid A-core oligosaccharide-O antigen repeating units.

P. aeruginosa is capable of coexpressing two distinct forms of LPS, designated A-band and B-band LPS, respectively. A-band LPS is a shorter, common form expressed by the majority of *P. aeruginosa* serotypes, and has a trisaccharide repeating unit of α-D-rhamnose linked 1→3, 1→3, 1→2. B-band LPS is the serotype-specific, O-antigen-containing form, and is a heteropolymer composed of di- to pentasaccharide repeats containing a wide variety of acyl sugars, amino sugars, and uronic acids. Both the A- and B-band repeating units are attached to lipid A-core, but there appear to be differences between them regarding point of attachment to and composition of the outer core region (Rivera et al., 1992).

The gene clusters for biosynthesis of core oligosaccharides/O-antigens *rfb* have been cloned and characterized from several bacterial species, including some from non-enteric genera such as *Bordetella* (Allen and Maskell, 1996), *Haemophilus* (Jarosik and Hansen, 1994), *Neisseria* (Gotschlich, 1994), *Vibrio* (Stroeher et al., 1992; Amor and Mutharia, 1995; Comstock et al., 1996), and *Xanthamonas* (Kingsley et al., 1993).

rfb clusters appear to be composed of mosaics of biosynthetic genes acquired horizontally from different sources (Reeves, 1993). Biochemical characterization of O-antigens from various species has shown that conservation of structure does not necessarily mirror conservation at the genetic level. Strains with identical O-antigens can

differ significantly in their *rfb* clusters, while unique O-antigens can be encoded by only slightly variant *rfb* genes in other strains (Whitfield and Valvano, 1993).

Lightfoot and Lam were the first to report the cloning of genes involved in the expression of A-band (Lightfoot and Lam, 1991) and B-band (Lightfoot and Lam, 1993) LPS of *P. aeruginosa*. A recombinant cosmid clone pFV3 complemented A-band LPS synthesis in an A-band-deficient mutant, rd7513. pFV3 also mediated A-band LPS synthesis in five of the six *P. aeruginosa* O serotypes which lack A-band LPS. Another cosmid clone, pFV100, complemented B-band LPS synthesis in mutant ge6, which lacks B-band LPS. Physical mapping of the genes involved in A-band and B-band LPS synthesis indicated that the two gene clusters are physically distinct and are separated by more than 1.9 Mbp on the *P. aeruginosa* PAO1 genome. A-band LPS genes mapped between 5.75 and 5.89 Mbp (10.5 to 13.3 min), and B-band LPS genes mapped at 1.9 Mbp (near 37 min) on the 5.9-Mbp chromosome.

The structure of the *P. aeruginosa* O5 O-antigen has been elucidated (Knirel et al., 1988). O5 has a trisaccharide repeating unit of 2-acetamido-3-acetamidino-2,3-dideoxy-D-mannuronic acid, 2,3-diacetamido-D-mannuronic acid, and *N*-acetyl-D-fucosamine (Figure 30). Serotypes O2, O16, O18, and O20 of *P. aeruginosa* have similar O-antigens to serotype O5, varying only in one linkage or one epimer from O5 (Knirel et al., 1988) (Figure 30). Immunochemical cross reactions have also been demonstrated among LPS of serotypes O2, O5 and O16 by the use of monoclonal antibodies (Lam et al., 1992). The *rfbA* (herein also referred to as "*psbL* " and "*wbp1*") from the O5 gene cluster has been characterized (Dasgupta and Lam, 1995). This O5 O-antigen biosynthetic gene has been shown to hybridize only with chromosomal DNA from the group of five serotypes with similar O-antigens, and not with the remaining fifteen serotypes.

There are currently three pathways proposed for biosynthesis and assembly of LPS, the Rfc-dependent and Rfc-independent pathways. Rfc is the O-antigen polymerase, and appears to be required for assembly of heteropolymeric O-antigens (Mäkelä and Stocker, 1984). In contrast, homopolymeric O-antigens appear to be assembled without an O-antigen polymerase (Whitfield, 1995). Rfc-dependent (or Wzy) LPS synthesis has been shown to involve at least two other gene products which act in concert with Rfc; RfbX (or Wzx), the putative flippase which translocates individual O-antigen units across the cytoplasmic membrane where they are polymerized by Rfc (or Wzy), and Rol (or Wzz), the regulator of O-antigen chain length, which determines the preferred O-antigen chain length characteristic of the individual strain or serotype (Batchelor et al., 1993; Bastin et al., 1993; Morona et al., 1994b; Dodgson et al., 1996).

SUMMARY OF THE INVENTION

The present inventors have characterized a *P. aeruginosa* B-band (*psb*) gene cluster involved in the synthesis and assembly of B-band lip polysaccharide i.e. O-antigen. The gene cluster is also known as and referred t herein as the *wbp* gene cluster.

The cluster contains two groups of genes, one of which is found in *P. aeruginosa* serotypes O2, O5, O16, O18, and O20, and the other is found in serotypes O1 to O20. The genes found in serotypes O2, O5, O16, O18, and O20 include the *psbL* gene also known as wbpL and rFA (Dasgupta and Lam, 1995), and the novel genes designated *rol*, *psbA*, *psbB*, *psbC psbD*, *psbE*, *rfc*, *psbF*, *psbG*, *psbH*, *psbI*, *psbJ*, and *psbK* ("Group I genes"), also known as and referred to herein as *wzz*, *wbpA*, *wbpB*, *wbpC*, *wbpD*, *wbpE*, *wzy*, *wbpF*, *wbpG*, *wbpH*, *wbpI*, *wbpJ*, and *wbpK* respectively. The genes found in serotypes O1 to O20 include the novel genes *psbM* and *psbN* which are also known as and referred to herein as *wbpM* and *wbpN* respectively ("Group II genes"). The *psb* gene cluster also contains genes which are not involved in LPS synthesis including the genes rpsA and himD and the nov l genes designated *uvrB*, insertion element *IS407*, *hisH and hisF*. The arrangement of the genes in the *wbp* gene cluster is shown in Figure 1.

The identification and sequencing of the genes and proteins in the *wbp* gene cluster permits the identification of substances which affect O-antigen synthesis or assembly in *P. aeruginosa*. These substances may be useful in inhibiting O-antigen synthesis or assembly thereby rendering the microorganisms more susceptible to attack by host defence mechanisms.

Broadly stated the present invention relates to an isolated *P. aeruginosa* B-band gene cluster containing the following genes:*rol (wzz)*, *psbA (wbpA)*, *psbB (wbpB)*, *psbC (wbpC)*, *psbD (wbpD)*, *psbE (wbpE)*, *rfc (wzy)*, *psbF (wbpF)*, *psbG (wbpG)*, *psbH (wbpH)*, *psbI (wbpI)*, *psbJ (wbpJ)*, *psbK (wbpK)*, *psbL (wbpL)*, *psbM (wbpM)*, and *psbN (wbpN)* involved in the synthesis, and assembly of lipopolysaccharide in *P. aeruginosa*. The terms in parenthesis correspond to other designations that have been given to these genes. The gene cluster may also contain the non-LPS gene *uvrB*, the insertion element *IS407 (IS1209)*, the genes *hisH* and *hisF* involved in histidine synthesis, the gene *rpsA* which encodes a 30 S ribosomal subunit protein S1 and the gene *himD* which encodes an integration host factor.

The present invention also relates to nucleic acid molecules encoding the following proteins: (1) (a) Rol (also known as Wzz); (b) PsbA (also known as WbpA); (c) PsbB (also known as WbpB); (d) PsbC (also known as WbpC); (e) PsbD (also known as WbpD); (f) PsbE (also known as WbpE); (g) Rfc (also known as Wzy); (h) PsbF (also known as WbpF); (i) PsbG (also known as WbpG); (j) PsbI (also known as WbpI); (k) PsbJ (also known as WbpJ); (l) PsbK (also known as WbpK); (m) PsbM (also known as WbpM); (n) PsbH (also known as WbpH) or (o) PsbN (also known as WbpN), involved in *P. aeruginosa* O-

antigen synthesis and assembly; (2) UvrB involved in ultraviolet repair; (3) HisH or HisF involved in histidine synthesis, or (4) RpsA a 30S ribosomal subunit protein S1. In addition, nucleic acid molecules are provided which contain sequences encoding two or more f the following proteins (1) (a) Rol (also known as Wzz); (b) PsbA (also known as WbpA); (c) PsbB (also known as WbpB); (d) PsbC (also known as WbpC); (e) PsbD (also known as WbpD); (f) PsbE (also known as WbpE); (g) Rfc (also known as Wzy); (h) PsbF (also known as WbpF); (i) HisH; (j) HisF; (k) PsbG (also known as WbpG); (l) PsbI (also known as WbpI); (m) PsbJ (also known as WbpJ); (n) PsbK (also known as WbpK); (o) PsbM (also known as WbpM); (p) PsbN (also known as WbpN); (q) PsbH (also known as WbpH); (r) PsbL (also known as WbpL); and (s) RpsA.

The invention also contemplates a nucleic acid molecule comprising a sequence encoding a truncation of a protein of the invention, an analog, or a homolog of a protein of the invention, or a truncation thereof.

The nucleic acid molecules of the invention may be inserted into an appropriate expression vector, i.e. a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Accordingly, recombinant expression vectors adapted for transformation of a host cell may be constructed which comprise a nucleic acid molecule of the invention and one or more transcription and translation elements operatively linked to the nucleic acid molecule.

The recombinant expression vector may be used to prepare transformed host cells expressing a protein of the invention. Therefore, the invention further provides host cells containing a recombinant molecule of the invention.

The invention further provides a method for preparing a protein of the invention utilizing the purified and isolated nucleic acid molecules of the invention. In an embodiment a method for preparing a protein of the invention is provided comprising (a) transferring a recombinant expression vector of the invention into a host cell; (b) selecting transformed host cells from untransformed host cells; (c) culturing a selected transformed host cell under conditions which allow expression of the protein; and (d) isolating the protein.

The invention further broadly contemplates an isolated protein characterized in that it has part or all of the primary structural conformation (ie. continuous sequence of amino acid residues) of a novel protein encoded by a gene of the *wbp* gene cluster of the invention. In an embodiment of the invention, a purified protein is provided which has the amino acid sequence as shown in Figure 3 or SEQ ID NO:2;, Figure 4 or SEQ ID NO:3; Figure 5 or SEQ ID NO:4; Figure 6 or SEQ ID NO:5; Figure 7 or SEQ ID NO:6; Figure 8 or SEQ ID NO:7; Figure 9 or SEQ ID NO:8; Figure 10 or SEQ ID NO:9; Figure 11 or SEQ ID NO:10; Figure 12 or SEQ ID NO:11; Figure 13 or SEQ ID NO:12; Figure 14 or

SEQ ID NO:13; Figure 15 or SEQ ID NO:14; Figure 16 or SEQ ID NO:15; Figure 17 or SEQ ID NO:16; or, Figure 18 or SEQ ID NO:17; Figure 19 or SEQ.ID. N .: 18; or, Figure 20 or SEQ.ID. N .: 19. The invention also includes truncations of the protein and analogs, homologs, and isoforms of the protein and truncations thereof.

The proteins of the invention may be conjugated with other molecules, such as proteins, to prepare fusion proteins. This may be accomplished, for example, by the synthesis of N-terminal or C-terminal fusion proteins.

The nucleic acid molecules of the invention allow those skilled in the art to construct nucleotide probes for use in the detection of nucleotide sequences in samples such as biological (e.g clinical specimens), food, or environmental samples. The nucleotide probes may also be used to detect nucleotide sequences that encode proteins related to or analogous to the proteins of the invention.

Accordingly, the invention provides a method for detecting the presence of a nucleic acid molecule having a sequence encoding a protein of the invention, comprising contacting the sample with a nucleotide probe which hybridizes with the nucleic acid molecule, to form a hybridization product under conditions which permit the formation of the hybridization product, and assaying for the hybridization product.

The invention further provides a kit for detecting the presence of a nucleic acid molecule having a sequence encoding a protein of the invention, comprising a nucleotide probe which hybridizes with the nucleic acid molecule, reagents required for hybridization of the nucleotide probe with the nucleic acid molecule, and directions for its use.

The nucleic acid molecules of the invention also permit the identification and isolation, or synthesis, of nucleotide sequences which may be used as primers to amplify a nucleic acid molecule of the invention, for example in the polymerase chain reaction (PCR).

Accordingly, the invention relates to a method of determining the presence of a nucleic acid molecule having a sequence encoding a protein of the invention in a sample, comprising treating the sample with primers which are capable of amplifying the nucleic acid molecule in an amplification reaction, preferably in a polymerase chain reaction, to form amplified sequences, under conditions which permit the formation of amplified sequences, and, assaying for amplified sequences.

The invention further relates to a kit for determining the presence of a nucleic acid molecule having a sequence encoding a protein of the invention in a sample, comprising primers which are capable of amplifying the nucleic acid molecule in an amplification reaction, preferably a polymerase chain reaction, to form amplified sequences, reagents required for amplifying the nucleic acid molecule thereof in the

amplification reaction, means for assaying the amplified sequences, and directions for its use.

The invention also relates to an antibody specific for an epitope of a protein of the invention, and methods for preparing the antibodies. Antibodies specific for a protein encoded by a Group I gene can be used to detect *P. aeruginosa* serotypes O2, O5, O16, O18, and O20 in a sample, and antibodies specific for a protein encoded by a Group II gene can be used to detect *P. aeruginosa* serotypes O1 to O20 in a sample. Therefore, the invention also relates to a method for detecting *P. aeruginosa* serotypes O2, O5, O16, O18, and O20 in a sample comprising contacting a sample with an antibody specific for an epitope of a protein encoded by a Group I gene which antibody is capable of being detected after it becomes bound to a protein in the sample, and assaying for antibody bound to protein in the sample, or unreacted antibody. A method is also provided for detecting *P. aeruginosa* serotypes O1 to O20 in a sample comprising contacting a sample with an antibody specific for an epitope of a protein encoded by a Group II gene which antibody is capable of being detected after it becomes bound to a protein in the sample, and assaying for antibody bound to protein in the sample, or unreacted antibody.

A kit for detecting *P. aeruginosa* serotypes in a sample comprising an antibody of the invention, preferably a monoclonal antibody and directions for its use is also provided. The kit may also contain reagents which are required for binding of the antibody to the protein in the sample.

As discussed above, the identification and sequencing of genes in the *wbp* gene cluster in *P. aeruginosa* permits the identification of substances which affect the activity of the proteins encoded by the genes in the cluster, or the expression of the proteins, thereby affecting O-antigen synthesis or assembly. These substances may be useful in rendering the microorganisms more susceptible to attack by host defence mechanisms. Accordingly, the invention provides a method for assaying for a substance that affects one or both of *P. aeruginosa* O-antigen synthesis or assembly comprising mixing a protein or nucleic acid molecule of the invention with a test substance which is suspected of affecting *P. aeruginosa* O-antigen synthesis or assembly, and determining the effect of the substance by comparing to a control.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF DRAWINGS

The inventi n will now be described in relation to the drawings:

Figure 1 shows the organization of the *P. aeruginosa* PAO1 *psb (wbp)* gene cluster;

Figure 2 shows the nucleic acid sequence of the *P. aeruginosa* PAO1 gene cluster (SEQ. ID. NO. 1);

Figure 3 shows the amino acid sequence of the Rol protein of the invention (SEQ. ID NO. 2);

Figure 4 shows the amino acid sequence of the PsbA (WbpA) protein of the invention (SEQ. ID NO. 3);

Figure 5 shows the amino acid sequence of the PsbB (WbpB) protein of the invention (SEQ. ID NO. 4);

Figure 6 shows the amino acid sequence of the PsbC (WbpC) protein of the invention (SEQ. ID NO. 5);

Figure 7 shows the amino acid sequence of the PsbD (WbpD) protein of the invention (SEQ. ID NO. 6);

Figure 8 shows the amino acid sequence of the PsbE (WbpE) protein of the invention (SEQ. ID NO. 7);

Figure 9 shows the amino acid sequence of the Rfc (Wzy) protein of the invention (SEQ. ID NO. 8);

Figure 10 shows the amino acid sequence of the PsbF (WbpF) protein of the invention (SEQ. ID NO. 9);

Figure 11 shows the amino acid sequence of the HisH protein of the invention (SEQ. ID NO. 10);

Figure 12 shows the amino acid sequence of the HisF protein of the invention (SEQ. ID NO. 11);

Figure 13 shows the amino acid sequence of the PsbG (WbpG) protein of the invention (SEQ. ID NO. 12);

Figure 14 shows the amino acid sequence of the PsbH (WbpH) protein of the invention (SEQ. ID NO. 13);

Figure 15 shows the amino acid sequence of the PsbI (WbpI) protein of the invention (SEQ. ID NO. 14);

Figure 16 shows the amino acid sequence of the PsbJ (WbpJ) protein of the invention (SEQ. ID NO. 15);

Figure 17 shows the amino acid sequence of the PsbK (WbpK) protein of the invention (SEQ. ID NO. 16);

Figure 18 shows the amino acid sequence of the PsbM (WbpM) protein of the invention (SEQ. ID NO. 17);

Figure 19 shows the amino acid sequence of the PsbN (WbpN) protein of the invention (SEQ. ID NO. 18);

Figure 20 shows the amino acid sequence of the UvrB protein of the invention (SEQ. ID NO. 19);

Figure 21 shows the amino acid sequence of PsbL (SEQ. ID NO. 20) (WbpL);

Figure 22 shows a silver-stained SDS-PAGE gel of LPS from PAO1, AK14O1, AK14O1(pFV100), and AK14O1(pFV.TK8) (Panel A) and Western immunoblots of this LPS reacted with O5-specific MAb MF15-4 (Panel B);

Figure 23 shows restriction maps of the chromosomal inserts from pFV100 and several pFV subclones, and the results of complementation studies of the SR mutants AK14O1 and rd7513 with the pFV subclones are also shown;

Figure 24 shows a Southern analysis of the three *rfc (wzy)* chromosomal mutants, OP5.2, OP5.3, and OP5.5, showing the insertion of an 875 bp Gm^R cassette into the *rfc (wzy)* gene (panel C), and restriction maps of the PAO1 wild-type (panel A) and mutant (panel B) *rfc (wzy)* coding regions are shown;

Figure 25 shows a silver-stained SDS-PAGE gel (panel A) and Western blots of LPS from PAO1, AK14O1 and the three *rfc (wzy)* chromosomal mutants, OP5.2, OP5.3, and OP5.5 (Panels B and C); and

Figure 26 shows the restriction maps of recombinant plasmids pFV161, pFV401, and pFV402;

Figure 27 are blots of Southern hybridizations of chromosomal DNA from PAO1 (lane 2) and *rol (wzz)* mutants (lanes 3 and 4);

Figure 28 are Western immunoblots showing the characterization of LPS from PAO1 and PAO1 *rol (wzz)* chromosomal mutants;

Figure 29 is an autoradiogram showing ^{35}S-labeled proteins expressed by pFV401, which contains the *rol (wzz)* gene and corresponding control plasmid vector pBluescript II SK in *E. coli* JM 109DE3 by use of the T7 expression system;

Figure 30 is a diagram showing the structures of the O-antigens of *P. aeruginosa* serotypes related to O5;

Figure 31 shows *E. coli* σ^{70} and similar regions in *psbA (wpbA), hisH, psbG (wpbG),* IS*407* and *psbN (wpbN);*

Figure 32 shows features of the *psb* genes of the *psb* gene cluster identifying the presumed start codon and spaces between RBS (ribosome binding sequence) and the first codon;

Figure 33 shows the sequences of the NAD-binding domains of PsbA, PsbK, and PsbM aligned with those of other bacterial proteins involved in polysaccharide biosynthesis;

Figure 34 shows a sequence alignment for PsbA (WpbA), *E. coli* RffD, and *B. solanaceraeum* EpsD;

Figure 35 shows a sequence alignment for PsbD (WpbD) and *Bordetella pertussis* BplB, CysE of a number of bacteria;

Figure 36 shows a sequence alignment for PsbE (WpbE) and BP-BplC, BS-DegT, S-EryC1, S-DnrJ, and BS-SpsC;

Figure 37 shows a hydropathy index computation for sequence PsbF;

Figure 38 shows a sequence alignment for PA-PsbI, BP-BplD, EC-NfrC, BS-OrfX, and SB-RfbC;

Figure 39 shows a sequence alignment for PA-PsbJ, BP-BplE, and YE-TrsE;

Figure 40 shows a sequence alignment for PA-PsbL, YE-TrsF and HI-Rfe;

Figure 41 shows a sequence alignment for PsbM, TrsG, BP-BplL, and SA-CapD;

Figure 42 shows the nucleotide sequence of the *rol (wzz)* gene;

Figure 43 is a physical map of the 5' end of the wbp cluster;

Figure 44 is a comparison of hydropathy plots of selected Wzz-like proteins;

Figure 45 shows the expression of *P. aeruginosa* Wzz in vitro;

Figure 46A shows an SDS-PAGE gel of LPS from Wzz knockout mutants;

Figure 46B shows a western immunoblot using Mab 18-19;

Figure 46C shows a western immunoblot using Mab MF15-4;

Figure 47 shows the ability of *P. aeruginosa* 05 Wzz to function in *E. Coli*;

Figure 48 shows an SDS-PAGE gel from WbpF knockout mutants;

Figure 49 shows the amino acid and nucleotide sequence encoding Rps A; and

Figure 50 shows the amino acid and nucleotide sequence encoding Him D.

DETAILED DESCRIPTION OF THE INVENTION

The following standard abbreviations for the amino acid residues are used throughout the specification: A, Ala - alanine; C, Cys - cysteine; D, Asp- aspartic acid; E, Glu - glutamic acid; F, Phe - phenylalanine; G, Gly - glycine; H, His - histidine; I, Ile -

isoleucine; K, Lys - lysine; L, Leu - leucine; M, Met - methionine; N, Asn - asparagine; P, Pro - proline; Q, Gln - glutamine; R, Arg - arginine; S, Ser - serine; T, Thr - threonine; V, Val - valine; W, Trp- tryptophan; Y, Tyr - tyrosine; and p.Y., P.Tyr - phosphotyr sine.

I. Nucleic Acid Molecules of the Invention

As hereinbefore mentioned, the present invention relates to an isolated *P. aeruginosa* B-band gene cluster containing genes involved in the synthesis and assembly of O-antigen in *P. aeruginosa*. The present invention also relates to the isolated genes which comprise the cluster.

The term "isolated" refers to a nucleic acid substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or chemical precursors, or other chemicals when chemically synthesized. The term "nucleic acid" is intended to include DNA and RNA and can be either double stranded or single stranded.

The *P. aeruginosa* B-band gene cluster comprises the following genes: *rol (wzz), psbA (wbpA), psbB (wbpB), psbC (wbpC), psbD (wbpD), psbE (wbpE), rfc (wzy), psbF (wbpF), psbG (wbpG), psbH (wbpH), psbI (wbpI), psbJ (wbpJ), psbK (wbpK), psbL (wbpL), psbM (wbpM),* and *psbN (wbpN)* involved in the synthesis, and assembly of lipopolysaccharide in *P. aeruginosa*. The gene cluster may also contain the non-LPS genes *hisH, hisF, himD, rspa, uvrB*, and the insertion element *IS407 (IS1209)*.

The genes preferably have the organization as shown in Figure 1 (SEQ. ID. NO. 1). In Figure 1, the genes necessary for sugar biosynthesis (Man(2NAc3N)A and Man(2NAc3NAc) biosynthesis) are scattered throughout the gene cluster (*wpbI (psbI), wpbE (psbE), wpbD (psbD), wpbB (psbB), wpbC (psbC*). The genes encoding transferases are interspersed throughout the *wpb (psb)* cluster (*wpbH (psbH), wpbJ (psbJ), wpbL, (wpbL)*), and are separated from one another by one gene each. The gene encoding the putative first transferase (Wpb (PsbL)), thought to initiate O-antigen assembly by attachment of an FucNAc residue to undecaprenol, is the most distal.

The invention provides nucleic acid molecules encoding the following proteins: (1) (a) Rol (Wzz); (b) PsbA (WbpA); (c) PsbB (WbpB); (d) PsbC (WbpC); (e) PsbD (WbpD); (f) PsbE (WbpE); (g) Rfc (Wzy); (h) PsbF (WbpF); (i) PsbG (WbpG); (j) PsbI (WbpI); (k) PsbJ (WbpJ); (l) PsbK (WbpK); (m) PsbM (WbpM); (n) PsbH (WbpH); and (o) PsbN (WbpN) involved in *P. aeruginosa* O-antigen synthesis and assembly; (2) UvrB involved in ultraviolet repair; (3) HisH or HisF involved in histidine synthesis or (4) himD involved in host factor integration and (5) RpsA a 30S ribosomal subunit protein S1. In addition, nucleic acid molecules are provided which contain sequences encoding two or more of the following proteins (1) (a) Rol (wzz); (b) PsbA (WbpA); (c) PsbB (WbpB); (d) PsbC (WbpC); (e) PsbD (WbpD); (f) PsbE (WbpE); (g) Rfc (Wzy); (h) PsbF (WbpF); (i) HisH; (j)

HisF; (k) PsbG (WbpG); (l) PsbI (WbpI); (m) PsbJ (WbpJ); (n) PsbK (WbpK); (o) PsbM (WbpM); (p) PsbN (WbpN); (q) PsbH (WbpH); (r) PsbL (WbpL); (s) RpsA or (t) HimD.

In an embodiment of the invention, an isolated nucleic acid molecule is provided having a sequence which encodes a protein having an amino acid sequence as shown in Figure 3 or SEQ.ID. No.: 2; Figure 4 or SEQ.ID. No.: 3; Figure 5 or SEQ.ID. No.: 4; Figure 6 or SEQ.ID. No.: 5; Figure 7 or SEQ.ID. No.: 6; Figure 8 or SEQ.ID. No.: 7; Figure 9 or SEQ.ID. No.: 8; Figure 10 or SEQ.ID. No.: 9; Figure 11 or SEQ.ID. No.: 10; Figure 12 or SEQ.ID. No.: 11; Figure 13 or SEQ.ID. No.: 12; Figure 14 or SEQ.ID. No.: 13; Figure 15 or SEQ.ID. No.: 14; Figure 16 or SEQ.ID. No.: 15; Figure 17 or SEQ.ID. No.: 16.; Figure 18 or SEQ.ID. No.: 17; Figure 19 or SEQ.ID. No.: 18; and Figure 20 or SEQ.ID. No.: 19.

Preferably, the purified and isolated nucleic acid molecule comprises

(a) a nucleic acid sequence containing nucleotides 1-479; 1286-2596; 2670-3620; 3689-5578; 5575-6066; 6152-6982; 7236-8552; 8549-9499; 9831-10388; 10388-11143; 11281-12411; 12427-13548; 13545-14633; 14651-15892; 15889-16851; 17935-19144; 19678-21675; 22302-23693; or 23704-24417, as shown in Figure 2 or SEQ. ID. NO.: 1, wherein T can also be U;

(b) a nucleic acid sequence containing two or more of nucleotides 1-479; 1286-2596; 2670-3620; 3689-5578; 5575-6066; 6152-6982; 7236-8552; 8549-9499; 9830-10388; 10388-11143; 11281-12411; 12427-13548; 13545-14633; 14651-15892; 15889-16851; 17935-19144; 19678-21675; 22302-23693; or 23704-24417, as shown in Figure 2 or SEQ. ID. NO.: 1, wherein T can also be U;

(c) nucleic acid sequences complementary to (a) or (b);

(d) nucleic acid sequences which are homologous to (a) or (b);

(e) a fragment of (a) to (d) that is at least 15 bases, preferably 20 to 30 bases, and which will hybridize to (a) to (d) under stringent hybridization conditions; or

(f) a nucleic acid molecule differing from any of the nucleic acids of (a) to (c) in codon sequences due to the degeneracy of the genetic code.

Specific embodiments of the nucleic acid molecule of the invention include the following:

1. An isolated nucleic acid molecule characterized by having a sequence encoding a Rol (Wzz) protein of *P. aeruginosa* which regulates O-antigen linking. The nucleic acid molecule preferably encodes Rol having the amino acid sequence as shown in Figure 3 or SEQ.ID. No.: 2, and most preferably comprises nucleotides 1-479 as shown in Figure 2 or SEQ.ID. No.: 1, or a nucleotide sequence as shown in Figure 42, which shows the full length nucleotide sequence of the *rol* gene.

2. An isolated nucleic acid molecule characterized by having a sequence encoding a PsbA (WbpA) protein of *P. aeruginosa* which has dehydrogenase

activity. The nucleic acid molecule preferably encodes PsbA having the amino acid sequence as shown in Figure 4 or SEQ.ID. No.: 3, and most preferably comprises nucleotides 1286-2596 as shown in Figure 2 or SEQ.ID. No.: 1.

3. An isolated nucleic acid molecule characterized by having a sequence encoding a PsbB (WbpB) protein of *P. aeruginosa*. The nucleic acid molecule preferably encodes PsbB having the amino acid sequence as shown in Figure 5 or SEQ.ID. No.: 4, and most preferably comprises nucleotides 2670-3620 as shown in Figure 2 or SEQ.ID. No.: 1.

4. An isolated nucleic acid molecule characterized by having a sequence encoding a PsbC (WbpC) protein of *P. aeruginosa* which has acetyltransferase activity. The nucleic acid molecule preferably encodes PsbC having the amino acid sequence as shown in Figure 6 or SEQ.ID. No.: 5, and most preferably comprises nucleotides 3689-5578 as shown in Figure 2 or SEQ.ID. No.: 1.

5. An isolated nucleic acid molecule characterized by having a sequence encoding a PsbD (WbpD) protein of *P. aeruginosa* which has acetyltransferase activity. The nucleic acid molecule preferably encodes PsbD having the amino acid sequence as shown in Figure 7 or SEQ.ID. No.: 6, and most preferably comprises nucleotides 5575-6066 as shown in Figure 2 or SEQ.ID. No.: 1.

6. An isolated nucleic acid molecule characterized by having a sequence encoding a PsbE (WbpE) protein of *P. aeruginosa*. The nucleic acid molecule preferably encodes PsbE having the amino acid sequence as shown in Figure 8 or SEQ.ID. No.: 7, and most preferably comprises nucleotides 6152-6982 as shown in Figure 2 or SEQ.ID. No.: 1.

7. An isolated nucleic acid molecule characterized by having a sequence encoding a Rfc (Wzy) protein of *P. aeruginosa* which has O-polymerase activity. The nucleic acid molecule preferably encodes Rfc having the amino acid sequence as shown in Figure 9 or SEQ.ID. No.: 8, and most preferably comprises nucleotides 7236-8552 as shown in Figure 2 or SEQ.ID. No.: 1. The nucleic acid molecule may comprise nucleotides 7236 to 8552 where base 8059 is "G". The Rfc coding region has a lower mol.% G+C than the *P. aeruginosa* chromosomal average and it has similar amino acid composition and codon usage to that reported for other Rfc proteins. Using a novel gene-replacement vector, the present inventors were able to generate PAO1 chromosomal *rfc* mutants. These knockout mutants express LPS containing complete core plus one O-repeat unit, indicating that they are no longer producing a functional O-polymerase enzyme.

8. An isolated nucleic acid molecule characterized by having a sequence encoding a PsbF (WbpF) protein of *P. aeruginosa*. The nucleic acid molecule preferably encodes PsbF having the amino acid sequence as shown in Figure 10 or SEQ.ID.

No.: 9, and most preferably comprises nucleotides 8549-9499 as shown in Figure 2 or SEQ.ID. No.: 1.

9. An isolated nucleic acid molecule characterized by having a sequence encoding a PsbG (WbpG) protein of *P. aeruginosa*. The nucleic acid molecule preferably encodes PsbG having the amino acid sequence as shown in Figure 13 or SEQ.ID. No.: 12, and most preferably comprises nucleotides 11281-12411 as shown in Figure 2 or SEQ.ID. No.: 1.

The present inventors have inserted a gentamicin cassette into *psbG* which resulted in B-band deficient mutants of PA01.

10. An isolated nucleic acid molecule characterized by having a sequence encoding a PsbH (WbpH) protein of *P. aeruginosa* which has ManA transferase activity. The nucleic acid molecule preferably encodes PsbH having the amino acid sequence as shown in Figure 14 or SEQ.ID. No.: 13, and most preferably comprises nucleotides 12427-13548 as shown in Figure 2 or SEQ.ID. No.: 1. The present inventors have produced a psbH knockout mutant of PA01 which is B-band deficient.

11. An isolated nucleic acid molecule characterized by having a sequence encoding a PsbI (WbpI) protein of *P. aeruginosa* which converts UDP-N-acetylglucosamine to UDP-N-acetylmannosamine. The nucleic acid molecule preferably encodes PsbI having the amino acid sequence as shown in Figure 15 or SEQ.ID. No.: 14, and most preferably comprises nucleotides 13545-14633 as shown in Figure 2 or SEQ.ID. No.: 1.

12. An isolated nucleic acid molecule characterized by having a sequence encoding a PsbJ (WbpJ) protein of *P. aeruginosa* which has ManA transferase activity. The nucleic acid molecule preferably encodes PsbJ having the amino acid sequence as shown in Figure 16 or SEQ.ID. No.: 15, and most preferably comprises nucleotides 14651-15892 as shown in Figure 2 or SEQ.ID. No.: 1.

13. An isolated nucleic acid molecule characterized by having a sequence encoding a PsbK (WbpK) protein of *P. aeruginosa* which has dehydratase activity. The nucleic acid molecule preferably encodes PsbK having the amino acid sequence as shown in Figure 17 or SEQ.ID. No.: 16, and most preferably comprises nucleotides 15889-16851 as shown in Figure 2 or SEQ.ID. No.: 1.

14. An isolated nucleic acid molecule characterized by having a sequence encoding a PsbM (WbpM) protein of *P. aeruginosa* and having dehydrogenase activity. The nucleic acid molecule preferably encodes PsbM having the amino acid sequence as shown in Figure 18 or SEQ.ID. No.: 17, and most preferably comprises nucleotides 19678-21675 as shown in Figure 2 or SEQ.ID. No.: 1. PsbM knockout mutants do not produce LPS.

15. An isolated nucleic acid molecule characterized by having a sequence encoding a PsbN (WbpN) protein of *P. aeruginosa*. The nucleic acid molecule preferably encodes PsbN having the amino acid sequence as shown in Figure 19 or SEQ.ID. No.: 18, and most preferably comprises nucleotides 22302-23693 as shown in Figure 2 or SEQ.ID. No.: 1.

16. An isolated nucleic acid molecule characterized by having a sequence encoding a UvrB protein of *P. aeruginosa* which is involved in ultraviolet repair. The nucleic acid molecule preferably encodes UvrB having the amino acid sequence as shown in Figure 20 or SEQ.ID. No.: 19, and most preferably comprises nucleotides 23704-24417 as shown in Figure 2 or SEQ.ID. No.: 1.

17. An isolated nucleic acid molecule characterized by having a sequence encoding a RpsA protein for a 30S ribosomal subunit. The nucleic acid molecule preferably encodes RpsA having the amino acid sequence as shown in Figure 49.

18. An isolated nucleic acid molecule characterized by having a sequence encoding a HimD protein for a host integration factor. The nucleic acid molecule preferably encodes HimD having the amino acid sequence as shown in Figure 50.

In an embodiment of the invention, the nucleic acid molecule contains two genes from the gene cluster of the invention, preferably two genes which are adjacent in the gene cluster. For example, the present inventors have found that *rfc (wzy)* and *psbF (wbpF)* are cotranscribed and they are both required for B-band synthesis. If *psbF (wbpF)* is absent, both A and B synthesis are knocked out indicating that its gene product is required for expressor of A and B- band LPS onto the core oligosaccharide. Accordingly, the invention provides a nucleic acid molecule encoding a PsbF (WpbF) protein and an Rfc (Wzy) protein. Preferably a nucleic acid molecule comprising nucleotides 7239 to 9499 as shown in Figure 2 or SEQ.ID. No.: 1.

It will be appreciated that the invention includes nucleic acid molecules encoding truncations of the proteins of the invention, and analogs and homologs of the proteins of the invention and truncations thereof, as described below. It will further be appreciated that variant forms of the nucleic acid molecules of the invention which arise by alternative splicing of an mRNA corresponding to a cDNA of the invention are encompassed by the invention.

Further, it will be appreciated that the invention includes nucleic acid molecules comprising nucleic acid sequences having substantial sequence homology with the nucleic acid sequences containing nucleotides 1-479; 1286-2596; 2670-3620; 3689-5578; 5575-6066; 6152-6982; 7236-8552; 8549-9499; 9831-10388; 10388-11143; 11281-12411; 12427-13548; 13545-14633; 14651-15892; 15889-16851; 17935-19144; 19678-21675; 22302-23693; or 23704-24417, as shown in Figure 2 or SEQ. ID. NO.: 2 and fragments thereof. The term "sequences

having substantial sequence h mology" means those nucleic acid sequences which have slight or inconsequential sequence variations from these sequences, i.e. the sequences function in substantially the same manner to produce functionally equivalent proteins. The variations may be attributable to local mutations or structural modifications.

Nucleic acid sequences having substantial homology include nucleic acid sequences having at least 80-90%, preferably 90% identity with the nucleic acid sequence 1-479; 1286-2596; 2670-3620; 3689-5578; 5575-6066; 6152-6982; 7236-8552; 8549-9499; 9831-10388; 10388-11143; 11281-12411; 12427-13548; 13545-14633; 14651-15892; 15889-16851; 17935-19144; 19678-21675; 22302-23693; or 23704-24417, as shown in Figure 2 or SEQ. ID. NO.: 2. By way of example, it is expected that a sequence having 80% sequence homology with the DNA sequence encoding PsbM of the invention will provide a functional PsbM protein.

Another aspect of the invention provides a nucleic acid molecule, and fragments thereof having at least 15 bases, which hybridizes to the nucleic acid molecules of the invention under hybridization conditions, preferably stringent hybridization conditions. Appropriate stringency conditions which promote DNA hybridization are known to those skilled in the art, or may be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. For example, the following may be employed: 6.0 x sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0 x SSC at 50°C. The stringency may be selected based on the conditions used in the wash step. For example, the salt concentration in the wash step can be selected from a high stringency of about 0.2 x SSC at 50°C. In addition, the temperature in the wash step can be at high stringency conditions, at about 65°C.

Isolated and purified nucleic acid molecules having sequences which differ from the nucleic acid sequence shown in SEQ ID NO:1 or Figure 2, and the nucleic acid sequences 1-479; 1286-2596; 2670-3620; 3689-5578; 5575-6066; 6152-6982; 7236-8552; 8549-9499; 9831-10388; 10388-11143; 11281-12411; 12427-13548; 13545-14633; 14651-15892; 15889-16851; 17935-19144; 19678-21675; 22302-23693; or 23704-24417, as shown in Figure 2 or SEQ. ID. NO.: 1, due to degeneracy in the genetic code are also within the scope of the invention. Such nucleic acids encode functionally equivalent proteins (e.g., a PsbM (WpbM) protein having dehydrogenase activity) but differ in sequence from the above mentioned sequences due to degeneracy in the genetic code.

An isolated nucleic acid molecule of the invention which comprises DNA can be isolated by preparing a labelled nucleic acid probe based on all or part of the nucleic acid sequences containing nucleotides 1-479; 1286-2596; 2670-3620; 3689-5578; 5575-6066; 6152-6982; 7236-8552; 8549-9499; 9831-10388; 10388-11143; 11281-12411; 12427-13548; 13545-14633; 14651-15892; 15889-16851; 17935-19144; 19678-21675; 22302-23693; or 23704-24417, as shown in Figure 2 or SEQ. ID. NO.: 2, and using this labelled nucleic acid probe to

screen an appropriate DNA library (e.g. a cDNA or genomic DNA library). For example, a whole genomic library isolated from a microorganism, such as a serotype of *P. aeruginosa*, can be used to isolate a DNA encoding a novel protein of the invention by screening the library with the labelled probe using standard techniques. Nucleic acids isolated by screening of a cDNA or genomic DNA library can be sequenced by standard techniques.

An isolated nucleic acid molecule of the invention which is DNA can also be isolated by selectively amplifying a nucleic acid encoding a novel protein of the invention using the polymerase chain reaction (PCR) methods and cDNA or genomic DNA. It is possible to design synthetic oligonucleotide primers from the nucleic acid molecules containing the nucleotides 1-479; 1286-2596; 2670-3620; 3689-5578; 5575-6066; 6152-6982; 7236-8552; 8549-9499; 9831-10388; 10388-11143; 11281-12411; 12427-13548; 13545-14633; 14651-15892; 15889-16851; 17935-19144; 19678-21675; 22302-23693; or 23704-24417, as shown in Figure 2 or SEQ. ID. NO.: 2, for use in PCR. A nucleic acid can be amplified from cDNA or genomic DNA using these oligonucleotide primers and standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. It will be appreciated that cDNA may be prepared from mRNA, by isolating total cellular mRNA by a variety of techniques, for example, by using the guanidinium-thiocyanate extraction procedure of Chirgwin et al., Biochemistry, 18, 5294-5299 (1979). cDNA is then synthesized from the mRNA using reverse transcriptase (for example, Moloney MLV reverse transcriptase available from Gibco/BRL, Bethesda, MD, or AMV reverse transcriptase available from Seikagaku America, Inc., St. Petersburg, FL).

An isolated nucleic acid molecule of the invention which is RNA can be isolated by cloning a cDNA encoding a novel protein of the invention into an appropriate vector which allows for transcription of the cDNA to produce an RNA molecule which encodes a novel protein of the invention. For example, a cDNA can be cloned downstream of a bacteriophage promoter, (e.g. a T7 promoter) in a vector, cDNA can be transcribed *in vitro* with T7 polymerase, and the resultant RNA can be isolated by standard techniques.

A nucleic acid molecule of the invention may also be chemically synthesized using standard techniques. Various methods of chemically synthesizing polydeoxynucleotides are known, including solid-phase synthesis which, like peptide synthesis, has been fully automated in commercially available DNA synthesizers (See e.g., Itakura et al. U.S. Patent No. 4,598,049; Caruthers et al. U.S. Patent No. 4,458,066; and Itakura U.S. Patent Nos. 4,401,796 and 4,373,071).

Determination of whether a particular nucleic acid molecule encodes a novel protein of the invention may be accomplished by expressing the cDNA in an appropriate host cell by standard techniques, and testing the activity of the protein using

the methods as described herein. F r example, the activity of a putative PsbM protein may be tested by mixing with an appropriate substrate and assaying for dehydrogenase activity. A cDNA having the activity of a novel protein of the invention so isolated can be sequenced by standard techniques, such as dideoxynucleotide chain termination or Maxam-Gilbert chemical sequencing, to determine the nucleic acid sequence and the predicted amino acid sequence of the encoded protein.

The initiation codon and untranslated sequences of the nucleic acid molecules of the invention may be determined using currently available computer software designed for the purpose, such as PC/Gene (IntelliGenetics Inc., Calif.). Regulatory elements can be identified using conventional techniques. The function of the elements can be confirmed by using these elements to express a reporter gene which is operatively linked to the elements. These constructs may be introduced into cultured cells using standard procedures. In addition to identifying regulatory elements in DNA, such constructs may also be used to identify proteins interacting with the elements, using techniques known in the art.

The sequence of a nucleic acid molecule of the invention may be inverted relative to its normal presentation for transcription to produce an antisense nucleic acid molecule. Preferably, an antisense sequence is constructed by inverting a region preceding the initiation codon or an unconserved region. In particular, the nucleic acid sequences contained in the nucleic acid molecules of the invention or a fragment thereof, preferably one or more of the nucleic acid sequences shown in the Sequence Listing as SEQ. ID. NO. 1 and in Figure 2 (i.e. a nucleic acid molecule containing nucleotides 1-479; 1286-2596; 2670-3620; 3689-5578; 5575-6066; 6152-6982; 7236-8552; 8549-9499; 9831-10388; 10388-11143; 11281-12411; 12427-13548; 13545-14633; 14651-15892; 15889-16851; 17935-19144; 19678-21675; 22302-23693; or 23704-24417) may be inverted relative to their normal presentation for transcription to produce antisense nucleic acid molecules.

The antisense nucleic acid molecules of the invention or a fragment thereof, may be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed with mRNA or the native gene e.g. phosphorothioate derivatives and acridine substituted nucleotides. The antisense sequences may be produced biologically using an expression vector introduced into cells in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense sequences are produced under the control of a high efficiency regulatory region, the activity of which may be determined by the cell type into which the vector is introduced.

The invention also provides nucleic acids encoding fusion proteins comprising a novel protein of the invention and a selected protein, or a selectable marker protein (see below).

II. Novel Proteins of the Invention

The invention further broadly contemplates an isolated protein characterized in that it has part or all of the primary structural conformation (ie. continuous sequence of amino acid residues) of a novel protein encoded by a gene of the *psb* gene cluster of the invention. In an embodiment of the invention, an isolated protein is provided which has the amino acid sequence as shown in Figure 3 or SEQ ID NO:2; (Rol or Wzz), Figure 4 or SEQ ID NO:3 (PsbA or WbpA) Figure 5 or SEQ ID NO:4 (PsbB or WbpB); Figure 6 or SEQ ID NO:5 (PsbC or WbpC); Figure 7 or SEQ ID NO:6 (PsbD or WbpD); Figure 8 or SEQ ID NO:7 (PsbE or WbpE); Figure 9 or SEQ ID NO:8 (Rfc or Wzy); Figure 10 or SEQ ID NO:9 (PsbF or WbpF); Figure 11 or SEQ ID NO:10 (HisH); Figure 12 or SEQ ID NO:11 (HisF); Figure 13 or SEQ ID NO:12 (PsbG or WbpG); Figure 14 or SEQ ID NO:13 (PsbH or WbpH); Figure 15 or SEQ ID NO:14 (PsbI or WbpI); Figure 16 or SEQ ID NO:15 (PsbJ or WbpJ); Figure 17 or SEQ ID NO:16 (PsbK or WbpK); Figure 18 or SEQ ID NO:17 (PsbM or WbpM); Figure 19 or SEQ ID NO:18 (PsbN or WbpN); or Figure 20 or SEQ ID NO:19 (UvrB).

The gene products of *rol, psbA, psbB, psbC, psbD, psbE, rfc, psbF, hisH, hisF, psbG, psbH, psbI, psbJ, psbL,* and *psbK* (also known as *wzz, wbpA, wbpB, wbpC, wbpD, wbpE, wzy, wbpF, hisH, hisF, wbpG, wbpH, wpbI, wbpJ* respectively) are expected to be found in serotypes O2, O5, O16, O18, and O20, and the gene products of *psbM* and *psbN* (also known as *wbpM* and *wbpN*, respectively) are expected to be found in serotypes O1 to O20. The gene products of *hisF* and *hisH* are not found in serotype O6.

Specific embodiments of the invention include the following:

1. An isolated Rol (Wzz) protein of *P. aeruginosa* which regulates O-antigen linking, having the amino acid sequence as shown in Figure 3 or SEQ.ID. No.: 2. The function of Rol may be associated with the Rfc protein.

2. An isolated PsbA (WbpA) protein of *P. aeruginosa* which has dehydrogenase activity, and the amino acid sequence as shown in Figure 4 or SEQ.ID. No.: 3. PsbA may be involved in the biosynthesis of mannuronic acid residues.

3. An isolated PsbB (WbpB) protein of *P. aeruginosa* having the amino acid sequence as shown in Figure 5 or SEQ.ID. No.: 4. PsbB may be involved in Fuc2NAc biosynthesis.

4. An isolated PsbC (WbpC) protein of *P. aeruginosa* which has acetyltransferase activity and the amino acid sequence as shown in Figure 6 or SEQ.ID. No.: 5. PsbC may be involved in the acetylation of mannuronic acid residues in the O-antigen.

5. An isolated PsbD (WbpD) protein of *P. aeruginosa* which has acetyltransferase activity and the amino acid sequence as shown in Figure 7 or SEQ.ID. No.: 6. PsbD may be involved in the acetylation of mannuronic acid residues in the O-antigen.

6. An isolated PsbE (WbpE) protein of *P. aeruginosa*. having the amino acid sequence as shown in Figure 8 or SEQ.ID. No.: 7. PsbE may be involved in the biosynthesis of 2,3-, 2,4-, and 2,6-dideoxy sugars such as 2,3-dideoxy mannuronic acid produced by *P. aeruginosa* O5.

7. An isolated Rfc (Wzy) protein of *P. aeruginosa* which has O-polymerase activity and the amino acid sequence as shown in Figure 9 or SEQ.ID. No.: 8. The Rfc protein is characterized as very hydrophobic, and it is an integral membrane protein with 11 putative membrane spanning domains.

8. An isolated PsbF (WbpF) protein of *P. aeruginosa*. having the amino acid sequence as shown in Figure 10 or SEQ.ID. No.: 9. PsbF is translationally coupled with *rfc* and it is a putative flippase.

9. An isolated PsbG (WbpG) protein of *P. aeruginosa* which has the amino acid sequence as shown in Figure 13 or SEQ.ID. No.: 12.

10. An isolated PsbH (WbpH) protein of *P. aeruginosa* which has ManA transferase activity and the amino acid sequence as shown in Figure 14 or SEQ.ID. No.: 13. PsbH may be involved in the addition of ManA (i.e. Man(2NAc3N)A) to the O-antigen unit.

11. An isolated PsbI (WbpI) protein of *P. aeruginosa* which converts UDP-N-acetylglucosamine to UDP-N-acetylmannosamine, and has the amino acid sequence as shown in Figure 15 or SEQ.ID. No.: 14.

12. An isolated PsbJ (WbpJ) protein of *P. aeruginosa* which has ManA transferase activity, and the amino acid sequence as shown in Figure 16 or SEQ.ID. No.: 15. Based on their gene order and their relative hydropathic indices, the *psbJ* and *psbH* gene products are thought to transfer Man(NAc)2A and Man(2Nac3N)A, respectively.

13. An isolated PsbK (WbpK) protein of *P. aeruginosa* which has dehydratase activity, and the amino acid sequence as shown in Figure 17 or SEQ.ID. No.: 16.

14. An isolated PsbM (WbpM) protein of *P. aeruginosa* having dehydrogenase activity, and the amino acid sequence as shown in Figure 18 or SEQ.ID. No.: 17. PsbM is involved in the biosynthesis of N-acetylfucosamine residues of the O-antigen. PsbM contains 2 NAD binding domains.

15. An isolated PsbN (WbpN) protein of *P. aeruginosa*. having the amino acid sequence as shown in Figure 19 or SEQ.ID. No.: 18.

16. An UvrB protein of *P. aeruginosa* which is involved in ultraviolet repair and has the amino acid sequence as shown in Figure 20 or SEQ.ID. No.: 19.

The m lecular weights, isoelectric points, and hydropathic indices of the Rol (Wzz), PsbA (WbpA), PsbB (WbpB), PsbC (WbpC), PsbD (WbpD), PsbE (WbpE), Rfc (Wzy), PsbF (WbpF), PsbG (WbpG), PsbH (WbpH), PsbI (WbpI), PsbJ (WbpJ), PsbK (WbpK), PsbM (WbpM) and PsbN (WbpN) proteins are shown in Table 1.

Within the context of the present invention, a protein of the invention may include various structural forms of the primary protein which retain biological activity. For example, a protein of the invention may be in the form of acidic or basic salts or in neutral form. In addition, individual amino acid residues may be modified by oxidation or reduction.

In addition to the full length amino acid sequences (Figures 3 to 20 or SEQ. ID.NOS:2 to 19), the proteins of the present invention may also include truncations of the proteins, and analogs, and homologs of the proteins and truncations thereof as described herein. Truncated proteins may comprise peptides of at least fifteen amino acid residues.

The proteins of the invention may also include analogs of the proteins having the amino acid sequences shown in Figures 3 to 20, or SEQ.ID. NOS: 2 to 19 and/or truncations thereof as described herein, which may include, but are not limited to an amino acid sequence containing one or more amino acid substitutions, insertions, and/or deletions. Amino acid substitutions may be of a conserved or non-conserved nature. Conserved amino acid substitutions involve replacing one or more amino acids of the proteins of the invention with amino acids of similar charge, size, and/or hydrophobicity characterisitics. When only conserved substitutions are made the resulting analog should be functionally equivalent. Non-conserved substitutions involve replacing one or more amino acids of the amino acid sequence with one or more amino acids which possess dissimilar charge, size, and/or hydrophobicity characteristics.

One or more amino acid insertions may be introduced into the amino acid sequences shown in Figures 3 to 20, or SEQ.ID. NOS:2 to 19. Amino acid insertions may consist of single amino acid residues or sequential amino acids ranging from 2 to 15 amino acids in length. For example, amino acid insertions may be used to destroy target sequences so that the protein is no longer active. This procedure may be used *in vivo* to inhibit the activity of a protein of the invention.

Deletions may consist of the removal of one or more amino acids, or discrete portions from the amino acid sequences shown in Figures 3 to 20 or SEQ.ID. NOS:2 to 19. The deleted amino acids may or may not be contiguous. The lower limit length of the resulting analog with a deletion mutation is about 10 amino acids, preferably 100 amino acids.

Analogs of a protein of the invention may be prepared by introducing mutations in the nucleotide sequence encoding the protein. Mutations in nucleotide sequences constructed for expression of analogs of a protein of the invention must preserve the reading frame of the coding sequences. Furthermore, the mutations will preferably not create complementary regions that could hybridize to produce secondary mRNA structures, such as loops or hairpins, which could adversely affect translation of the receptor mRNA.

Mutations may be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

Alternatively, oligonucleotide-directed site specific mutagenesis procedures may be employed to provide an altered gene having particular codons altered according to the substitution, deletion, or insertion required. Deletion or truncation of a protein of the invention may also be constructed by utilizing convenient restriction endonuclease sites adjacent to the desired deletion. Subsequent to restriction, overhangs may be filled in, and the DNA religated. Exemplary methods of making the alterations set forth above are disclosed by Sambrook et al (Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, 1989).

The proteins of the invention also include homologs of the amino acid sequences shown in Figures 3 to 20, or SEQ.ID. NOS:2 to 19 and/or truncations thereof as described herein. Such homologs are proteins whose amino acid sequences are comprised of amino acid sequences that hybridize under stringent hybridization conditions (see discussion of stringent hybridization conditions herein) with a probe used to obtain a protein of the invention. Homologs of a protein of the invention will have the same regions which are characteristic of the protein.

Amino acid homologies for WbpA, WbpD, WbpE, HisH, HisF, WbpI, WbpJ, WbpK, WbpM and Wzz proteins are shown in Table 2 to 4. It will be appreciated that the invention includes WbpA, WbpD, WbpE, HisH, HisF, WbpI, WbpJ, WbpK, WbpM and Wzz proteins having at least 51%, 84%, 76%, 57%, 54%, 70%, 53%, 54%, 61% and 51% homology, respectively.

The invention also contemplates isoforms of the proteins of the invention. An isoform contains the same number and kinds of amino acids as a protein of the invention, but the isoform has a different molecular structure. The isoforms contemplated by the present invention are those having the same properties as a protein of the invention as described herein.

The present invention also includes a protein of the invention conjugated with a selected protein, or a selectable marker protein (see below) to produce

fusion proteins. Additionally, immunogenic portions of a protein of the invention are within the scope of the invention.

The proteins of the invention (including truncations, analogs, etc.) may be prepared using recombinant DNA methods. Accordingly, the nucleic acid molecules of the present invention having a sequence which encodes a protein of the invention may be incorporated in a known manner into an appropriate expression vector which ensures good expression of the protein. Possible expression vectors include but are not limited to cosmids, plasmids, or modified viruses (e.g. replication defective retroviruses, adenoviruses and adeno-associated viruses), so long as the vector is compatible with the host cell used. The expression vectors are "suitable for transformation of a host cell", means that the expression vectors contain a nucleic acid molecule of the invention and regulatory sequences selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid molecule. Operatively linked is intended to mean that the nucleic acid is linked to regulatory sequences in a manner which allows expression of the nucleic acid.

The invention therefore contemplates a recombinant expression vector of the invention containing a nucleic acid molecule of the invention, or a fragment thereof, and the necessary regulatory sequences for the transcription and translation of the inserted protein-sequence. Suitable regulatory sequences may be derived from a variety of sources, including bacterial, fungal, or viral genes (For example, see the regulatory sequences described in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990). Selection of appropriate regulatory sequences is dependent on the host cell chosen as discussed below, and may be readily accomplished by one of ordinary skill in the art. Examples of such regulatory sequences include: a transcriptional promoter and enhancer or RNA polymerase binding sequence, a ribosomal binding sequence, including a translation initiation signal. Additionally, depending on the host cell chosen and the vector employed, other sequences, such as an origin of replication, additional DNA restriction sites, enhancers, and sequences conferring inducibility of transcription may be incorporated into the expression vector. It will also be appreciated that the necessary regulatory sequences may be supplied by the native protein and/or its flanking regions.

The invention further provides a recombinant expression vector comprising a DNA nucleic acid molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression, by transcription of the DNA molecule, of an RNA molecule which is antisense to a nucleotide sequence comprising 1-479; 1293-2596; 2670-3620; 3277-5577; 5574-6065; 6151-6981; 7235-8551; 8548-9498; 9830-10388; 10388-11143; 11281-12411; 12427-13548; 13545-14633; 14651-15892; 15889-16851; 18032-19141; 19678-21675; 22302-23693; or 23704-24417, as shown in Figure 2 or SEQ. ID. NO.: 2. Regulatory sequences

operatively linked to the antisense nucleic acid can be chosen which direct the continuous expression of the antisense RNA m lecule.

The recombinant expression vectors of the invention may also contain a selectable marker gene which facilitates the selection of host cells transformed or transfected with a recombinant molecule of the invention. Examples of selectable marker genes are genes encoding a protein such as G418 and hygromycin which confer resistance to certain drugs, β-galactosidase, chloramphenicol acetyltransferase, or firefly luciferase. Transcription of the selectable marker gene is monitored by changes in the concentration f the selectable marker protein such as β-galactosidase, chloramphenicol acetyltransferase, or firefly luciferase. If the selectable marker gene encodes a protein conferring antibiotic resistance such as neomycin resistance transformant cells can be selected with G418. Cells that have incorporated the selectable marker gene will survive, while the other cells die. This makes it possible to visualize and assay for expression of recombinant expression vectors of the invention and in particular to determine the effect of a mutation on expression and phenotype. It will be appreciated that selectable markers can be introduced on a separate vector from the nucleic acid of interest.

The recombinant expression vectors may also contain genes which encode a fusion moiety which provides increased expression of the recombinant protein; increased solubility of the recombinant protein; and aid in the purification of a target recombinant protein by acting as a ligand in affinity purification. For example, a proteolytic cleavage site may be added to the target recombinant protein to allow separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Typical fusion expression vectors include pGEX (Amrad Corp., Melbourne, Australia), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-tranferase (GST), maltose E binding protein, or protein A, respectively, to the recombinant protein.

Recombinant expression vectors can be introduced into host cells to produce a transformant host cell. The term "transformant host cell" is intended to include prokaryotic and eukaryotic cells which have been transformed or transfected with a recombinant expression vector of the invention. The terms "transformed with", "transfected with", "transformation" and "transfection" are intended to encompass introduction of nucleic acid (e.g. a vector) into a cell by one of many possible techniques known in the art. Prokaryotic cells can be transformed with nucleic acid by, for example, electroporation or calcium-chloride mediated transformation. Nucleic acid can be introduced into mammalian cells via conventional techniques such as calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofectin, electroporation or microinjection. Suitable methods for transforming and transfecting host cells can be found in

Sambrook et al. (Molecular Cl ning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory press (1989)), and other laboratory textbooks.

Suitable host cells include a wide variety of prokaryotic and eukaryotic host cells. For example, the proteins of the invention may be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus), yeast cells or mammalian cells. Other suitable host cells can be found in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (199 1).

More particularly, bacterial host cells suitable for carrying out the present invention include *E. coli*, as well as many other bacterial species well known to one of ordinary skill in the art. Bacterial expression vectors preferably comprise a promoter which functions in the host cell, one or more selectable phenotypic markers, and a bacterial origin of replication. Representative promoters include the β-lactamase (penicillinase) and lactose promoter system (see Chang et al., Nature 275:615, 1978), the *trp* promoter (Nichols and Yanofsky, Meth in Enzymology 101:155, 1983) and the *tac* promoter (Russell et al., Gene 20: 231, 1982). Representative selectable markers include various antibiotic resistance markers such as the kanamycin or ampicillin resistance genes. Suitable expression vectors include but are not limited to bacteriophages such as lambda derivatives or plasmids such as pBR322 (see Bolivar et al., Gene 2:9S, 1977), the pUC plasmids pUC18, pUC19, pUC118, pUC119 (see Messing, Meth in Enzymology 101:20-77, 1983 and Vieira and Messing, Gene 19:259-268, 1982), and pNH8A, pNH16a, pNH18a, and Bluescript M13 (Stratagene, La Jolla, Calif.).

Yeast and fungi host cells suitable for carrying out the present invention include, but are not limited to *Saccharomyces cerevisae*, the genera *Pichia* or *Kluyveromyces* and various species of the genus *Aspergillus*. Examples of vectors for expression in yeast *S. cerivisae* include pYepSec1 (Baldari. et al., (1987) Embo J. 6:229-234), pMFa (Kurjan and Herskowitz, (1982) Cell 30:933-943), pJRY88 (Schultz et al., (1987) Gene 54:113-123), and pYES2 (Invitrogen Corporation, San Diego, CA). Protocols for the transformation of yeast and fungi are well known to those of ordinary skill in the art.(see Hinnen et al., PNAS USA 75:1929, 1978; Itoh et al., J. Bacteriology 153:163, 1983, and Cullen et al. (Bio/Technology 5:369, 1987).

The proteins of the invention may also be prepared by chemical synthesis using techniques well known in the chemistry of proteins such as solid phase synthesis (Merrifield, 1964, J. Am. Chem. Assoc. 85:2149-2154) or synthesis in homogenous solution (Houbenweyl, 1987, Methods of Organic Chemistry, ed. E. Wansch, Vol. 15 I and II, Thieme, Stuttgart).

III. Applications

Detecti n of Nucleic Acid Molecules, Antibodies, and Diagnostic Applications

The nucleic acid molecules of the invention, allow those skilled in the art to construct nucleotide probes for use in the detection of nucleotide sequences in a sample. A nucleotide probe may be labelled with a detectable marker such as a radioactive label which provides for an adequate signal and has sufficient half life such as ^{32}P, ^{3}H, ^{14}C or the like. Other detectable markers which may be used include antigens that are recognized by a specific labelled antibody, fluorescent compounds, enzymes, antibodies specific for a labelled antigen, and chemiluminescent compounds. An appropriate label may be selected having regard to the rate of hybridization and binding of the probe to the nucleotide to be detected and the amount of nucleotide available for hybridization.

The nucleotide probes may be used to detect genes that encode proteins related to or analogous to proteins of the invention.

Accordingly, the present invention also relates to a method of detecting the presence of nucleic acid molecules encoding a protein of the invention in a sample comprising contacting the sample under hybridization conditions with one or more of nucleotide probes which hybridize to the nucleic acid molecules and are labelled with a detectable marker, and determining the degree of hybridization between the nucleic acid molecule in the sample and the nucleotide probes.

In an embodiment of the invention a method for detecting *P. aeruginosa* serotypes 01 to 020 in a sample comprising contacting the sample with a nucleotide sequence encoding PsbM, or PsbN, or a fragment thereof, under conditions which permit the nucleic acid molecule to hybridize with a complementary sequence in the sample to form a hybridization product, and assaying for the hybridization product.

In another embodiment of the invention a method for detecting *P.aeruginosa* serotypes O2, O5, O16, O18, O20 in a sample comprising contacting the sample with a nucleotide sequence encoding one or more of RoI, PsbB, PsbC, PsbD, PsbE, rfc, PsbF, PsbG, PsbH, PsbI, PsbJ, PsbK (also known as Wzz, WbpB, WbpC, WbpD, WbpE, Wzy, WbpF, WbpG, WbpH, WbpI, WbpJ, WbpK, respectively), HisH, or HisF or a fragment thereof, under conditions which permit the nucleic acid molecule to hybridize with complementary sequences in the sample to form hybridization products, and assaying for the hybridization products.

Hybridization conditions which may be used in the methods of the invention are known in the art and are described for example in Sambrook J, Fritch EF, Maniatis T. In: Molecular Cloning, A Laboratory Manual,1989. (Nolan C, Ed.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. The hybridization product may be assayed using techniques known in the art. The nucleotide probe may be labelled with a

detectable marker as described herein and the hybridization product may be assayed by detecting the detectable marker or the detectable change produced by the detectable marker.

The nucleic acid molecule of the invention also permits the identification and isolation, or synthesis of nucleotide sequences which may be used as primers to amplify a nucleic acid molecule of the invention, for example in the polymerase chain reaction (PCR) which is discussed in more detail below. The primers may be used to amplify the genomic DNA of other bacterial species known to have LPS. The PCR amplified sequences can be examined to determine the relationship between the various LPS genes.

The length and bases of the primers for use in the PCR are selected so that they will hybridize to different strands of the desired sequence and at relative positions along the sequence such that an extension product synthesized from one primer when it is separated from its template can serve as a template for extension of the other primer into a nucleic acid of defined length.

Primers which may be used in the invention are oligonucleotides i.e. molecules containing two or more deoxyribonucleotides of the nucleic acid molecule of the invention which occur naturally as in a purified restriction endonuclease digest or are produced synthetically using techniques known in the art such as for example phosphotriester and phosphodiester methods (See Good et al Nucl. Acid Res 4:2157, 1977) or automated techniques (See for example, Conolly, B .A. Nucleic Acids Res. 15:15(7): 3131, 1987). The primers are capable of acting as a point of initiation of synthesis when placed under conditions which permit the synthesis of a primer extension product which is complementary to the DNA sequence of the invention i.e. in the presence of nucleotide substrates, an agent for polymerization such as DNA polymerase and at suitable temperature and pH. Preferably, the primers are sequences that do not form secondary structures by base pairing with other copies of the primer or sequences that form a hair pin configuration. The primer preferably contains between about 7 and 25 nucleotides.

The primers may be labelled with detectable markers which allow for detection of the amplified products. Suitable detectable markers are radioactive markers such as P-32, S-35, I-125, and H-3, luminescent markers such as chemiluminescent markers, preferably luminol, and fluorescent markers, preferably dansyl chloride, fluorcein-5-isothiocyanate, and 4-fluor-7-nitrobenz-2-axa-1,3 diazole, enzyme markers such as horseradish peroxidase, alkaline phosphatase, β-galactosidase, acetylcholinesterase, or biotin.

It will be appreciated that the primers may contain non-complementary sequences provided that a sufficient amount of the primer contains a

sequence which is complementary to a nucleic acid molecule of the invention or oligonucleotide fragment thereof, which is to be amplified. Restriction site linkers may also be incorporated into the primers allowing for digestion of the amplified products with the appropriate restriction enzymes facilitating cloning and sequencing of the amplified product.

In an embodiment of the invention a method of determining the presence of a nucleic acid molecule having a sequence encoding a protein of the invention is provided comprising treating the sample with primers which are capable of amplifying the nucleic acid molecule or a predetermined oligonucleotide fragment thereof in a polymerase chain reaction to form amplified sequences, under conditions which permit the formation of amplified sequences and, assaying for amplified sequences.

In a preferred embodiment of the invention, a method for detecting *P. aeruginosa* serotypes O1 to O20 in a sample is provided comprising treating the sample with a primer which is capable of amplifying nucleic acid molecules comprising nucleotide sequences encoding PsbM (WbpM), or PsbN (WbpN), or a predetermined oligonucleotide fragment thereof, in a polymerase chain reaction to form amplified sequences, under conditions which permit the formation of amplified sequences and, assaying for amplified sequences.

In another preferred embodiment of the invention, a method for detecting *P. aeruginosa* serotypes O2, O5, O16, O18, O20 in a sample is provided comprising treating the sample with a primer which is capable of amplifying nucleic acid molecules comprising nucleotide sequences encoding Rol, PsbA, PsbB, PsbC, PsbD, PsbE, Rfc, PsbF, PsbG, PsbH, PsbI, PsbJ, PsbK, (also known as Wzz, WbpA, WbpB, WbpC, WbpD, WbpE, Wzy, WbpF, WbpG, WbpH, WbpI, WbpJ, WbpK respectively) HisH or HisF, or a predetermined oligonucleotide fragment thereof, in a polymerase chain reaction to form amplified sequences, under conditions which permit the formation of amplified sequences and, assaying for amplified sequences.

The polymerase chain reaction refers to a process for amplifying a target nucleic acid sequence as generally described in Innis et al, Academic Press, 1990 in Mullis el al., U.S. Pat. No. 4,863,195 and Mullis, U.S. Patent No. 4,683,202 which are incorporated herein by reference. Conditions for amplifying a nucleic acid template are described in M.A. Innis and D.H. Gelfand, PCR Protocols, A Guide to Methods and Applications M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White eds, pp3-12, Academic Press 1989, which is also incorporated herein by reference.

The amplified products can be isolated and distinguished based on their respective sizes using techniques known in the art. For example, after amplification, the DNA sample can be separated on an agarose gel and visualized, after staining with

ethidium bromide, under ultra violet (UW) light. DNA may be amplified to a desired level and a further extension reaction may be performed to incorporate nucleotide derivatives having detectable markers such as radioactive labelled or biotin labelled nucleoside triphosphates. The primers may also be labelled with detectable markers as discussed above. The detectable markers may be analyzed by restriction and electrophoretic separation or other techniques known in the art.

The conditions which may be employed in the methods of the invention using PCR are those which permit hybridization and amplification reactions to proceed in the presence of DNA in a sample and appropriate complementary hybridization primers. Conditions suitable for the polymerase chain reaction are generally known in the art. For example, see M.A. Innis and D.H. Gelfand, PCR Protocols, A guide to Methods and Applications M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White eds, pp3-12, Academic Press 1989, which is incorporated herein by reference. Preferably, the PCR utilizes polymerase obtained from the thermophilic bacterium *Thermus aquatics* (Taq polymerase, GeneAmp Kit, Perkin Elmer Cetus) or other thermostable polymerase may be used to amplify DNA template strands.

It will be appreciated that other techniques such as the Ligase Chain Reaction (LCR) and NASBA may be used to amplify a nucleic acid molecule of the invention (Barney in "PCR Methods and Applications", August 1991, Vol.1(1), page 5, and European Published Application No. 0320308, published June 14, 1989, and U.S. Serial NO. 5,130,238 to Malek).

A protein of the invention can be used to prepare antibodies specific for the protein. Antibodies can be prepared which bind a distinct epitope in an unconserved region of the protein. An unconserved region of the protein is one which does not have substantial sequence homology to other proteins. Alternatively, a region from a well-characterized domain can be used to prepare an antibody to a conserved region of a protein of the invention. Antibodies having specificity for a protein of the invention may also be raised from fusion proteins.

Conventional methods can be used to prepare the antibodies. For example, by using a peptide of a protein of the invention, polyclonal antisera or monoclonal antibodies can be made using standard methods. A mammal, (e.g., a mouse, hamster, or rabbit) can be immunized with an immunogenic form of the peptide which elicits an antibody response in the mammal. Techniques for conferring immunogenicity on a peptide include conjugation to carriers or other techniques well known in the art. For example, the peptide can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassay procedures can be used with the immunogen as antigen to assess the levels of

antibodies. Following immunization, antisera can be obtained and, if desired, polyclonal antibodies isolated from the sera.

To pr duce monoclonal antibodies, antibody producing cells (lymphocytes) can be harvested from an immunized animal and fused with myeloma cells by standard somatic cell fusion procedures thus immortalizing these cells and yielding hybridoma cells. Such techniques are well known in the art, (e.g., the hybridoma technique originally developed by Kohler and Milstein (Nature 256, 495-497 (1975)) as well as other techniques such as the human B-cell hybridoma technique (Kozbor et al., Immunol. Today 4, 72 (1983)), the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al. Monoclonal Antibodies in Cancer Therapy (1985) Allen R. Bliss, Inc., pages 77-96), and screening of combinatorial antibody libraries (Huse et al., Science 246, 1275 (1989)]. Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with the peptide and the monoclonal antibodies can be isolated. Therefore, the invention also contemplates hybridoma cells secreting monoclonal antibodies with specificity for a protein of the invention.

The term "antibody" as used herein is intended to include fragments thereof which also specifically react with a protein, of the invention, or peptide thereof. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above. For example, F(ab')2 fragments can be generated by treating antibody with pepsin. The resulting F(ab')2 fragment can be treated to reduce disulfide bridges to produce Fab' fragments.

Chimeric antibody derivatives, i.e., antibody molecules that combine a non-human animal variable region and a human constant region are also contemplated within the scope of the invention. Chimeric antibody molecules can include, for example, the antigen binding domain from an antibody of a mouse, rat, or other species, with human constant regions. Conventional methods may be used to make chimeric antibodies containing the immunoglobulin variable region which recognizes the gene product of the genes of the *psb* cluster of the invention (See, for example, Morrison et al., Proc. Natl Acad. Sci. U.S.A. 81,6851 (1985); Takeda et al., Nature 314, 452 (1985), Cabilly et al., U.S. Patent No. 4,816,567; Boss et al., U.S. Patent No. 4,816,397; Tanaguchi et al., European Patent Publication EP171496; European Patent Publication 0173494, United Kingdom patent GB 2177096B).

Monoclonal or chimeric antibodies specifically reactive with a protein of the invention as described herein can be further humanized by producing human constant region chimeras, in which parts of the variable regions, particularly the conserved framework regions of the antigen-binding domain, are of human origin and only the hypervariable regions are of non-human origin. Such immunoglobulin molecules may be

made by techniques known in the art, (e.g., Teng et al., Proc. Natl. Acad. Sci. U.S.A., 80, 7308-7312 (1983); Kozbor et al., Immunology Today, 4, 7279 (1983); Olsson et al., Meth. Enzymol., 92, 3-16 (1982)), and PCT Publication WO92/06193 or EP 0239400). Humanized antibodies can also be commercially produced (Scotgen Limited, 2 Holly Road, Twickenham, Middlesex, Great Britain.)

Specific antibodies, or antibody fragments, reactive against proteins of the invention may also be generated by screening expression libraries encoding immunoglobulin genes, or portions thereof, expressed in bacteria with peptides produced from the nucleic acid molecules of the present invention. For example, complete Fab fragments, VH regions and FV regions can be expressed in bacteria using phage expression libraries (See for example Ward et al., Nature 341, 544-546: (1989); Huse et al., Science 246, 1275-1281 (1989); and McCafferty et al. Nature 348, 552-554 (1990)). In an embodiment of the invention, antibodies that bind to an epitope of a protein of the invention are engineered using the procedures described in N. Tout and J. Lam (Clinc. Diagn. Lab. Immunol. Vol. 4(2):147-155, 1997).

The antibodies may be labelled with a detectable marker including various enzymes, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, biotin, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include S-35, Cu-64, Ga-67, Zr-89, Ru-97, Tc-99m, Rh-105, Pd-109, In-111, I-123, I-125, I131, Re-186, Au-198, Au-199, Pb-203, At-211, Pb-212 and Bi-212. The antibodies may also be labelled or conjugated to one partner of a ligand binding pair. Representative examples include avidin-biotin and riboflavin-riboflavin binding protein. Methods for conjugating or labelling the antibodies discussed above with the representative labels set forth above may be readily accomplished using conventional techniques.

The antibodies reactive against proteins of the invention (e.g. enzyme conjugates or labeled derivatives) may be used to detect a protein of the invention in various samples, for example they may be used in any known immunoassays which rely on the binding interaction between an antigenic determinant of a protein of the invention and the antibodies. Examples of such assays are radioimmunoassays, enzyme immunoassays (e.g. ELISA), immunofluorescence, immunoprecipitation, latex agglutination, hemagglutination, and histochemical tests. Thus, the antibodies may be used to identify or quantify th amount of a protein of the invention in a sample in order to diagnose *P. aeruginosa* infections.

A sample may be tested for the presence or absence of *P. aeruginosa* serotypes O1 to O20 by contacting the sample with an antibody specific for an epitope of PsbM (WbpM) or PsbN (WbpN) which antibody is capable of being detected after it becomes bound to PsbM (WbpM) or PsbN (WbpN) in the sample, and assaying for antibody bound to PsbM (WbpM) or PsbN (WbpN) in the sample, or unreacted antibody. A sample may also be tested for the presence or absence of *P. aeruginosa* serotypes O2, O5, O16, O18, and O20 by contacting the sample with an antibody specific for an epitope of a Rol, PsbA, PsbB, PsbC, PsbD, PsbE, Rfc, PsbF, PsbG, PsbH, PsbI, PsbJ, PsbK (also known as Wzz, WbpA, WbpB, WbpC, WbpD, WbpE, Wzy, WbpF, WbpG, WbpH, WbpI, WbpJ, WbpK respectively), HisH or HisF, protein which antibody is capable of being detected after it becomes bound to the protein in the sample, and assaying for antibody bound to protein in the sample, or unreacted antibody.

In a method of the invention a predetermined amount of a sample or concentrated sample is mixed with antibody or labelled antibody. The amount of antibody used in the process is dependent upon the labelling agent chosen. The resulting protein bound to antibody or labelled antibody may be isolated by conventional isolation techniques, for example, salting out, chromatography, electrophoresis, gel filtration, fractionation, absorption, polyacrylamide gel electrophoresis, agglutination, or combinations thereof.

The sample or antibody may be insolubilized, for example, the sample or antibody can be reacted using known methods with a suitable carrier. Examples of suitable carriers are Sepharose or agarose beads. When an insolubilized sample or antibody is used protein bound to antibody or unreacted antibody is isolated by washing. For example, when the sample is blotted onto a nitrocellulose membrane, the antibody bound to a protein of the invention is separated from the unreacted antibody by washing with a buffer, for example, phosphate buffered saline (PBS) with bovine serum albumin (BSA).

When labelled antibody is used, the presence of a *P. aeruginosa* serotype can be determined by measuring the amount of labelled antibody bound to a protein of the invention in the sample or of the unreacted labelled antibody. The appropriate method of measuring the labelled material is dependent upon the labelling agent.

When unlabelled antibody is used in the method of the invention, the presence of a *P. aeruginosa* serotype can be determined by measuring the amount of antibody bound to the *P. aeruginosa* serotype using substances that interact specifically with the antibody to cause agglutination or precipitation. In particular, labelled antibody against an antibody specific for a protein of the invention, can be added to the reaction mixture. The presence of a *P. aeruginosa* serotype can be determined by a suitable method from among the already described techniques depending on the type of labelling agent. The antibody against an antibody specific for a protein of the invention can be prepared and labelled by

conventional procedures known in the art which have been described herein. The antibody against an antibody specific for a protein of the invention may be a species specific anti-immunoglobulin antibody or monoclonal antibody, for example, goat anti-rabbit antibody may be used to detect rabbit antibody specific for a protein of the invention.

The reagents suitable for applying the methods of the invention may be packaged into convenient kits providing the necessary materials, packaged into suitable containers. Such kits may include all the reagents required to detect a *P. aeruginosa* serotype in a sample by means of the methods described herein, and optionally suitable supports useful in performing the methods of the invention.

In one embodiment of the invention the kit contains a nucleotide probe which hybridizes with a nucleic acid molecule of the invention, reagents required for hybridization of the nucleotide probe with the nucleic acid molecule, and directions for its use. In another embodiment of the invention the kit includes antibodies of the invention and reagents required for binding of the antibody to a protein specific for a *P.aeruginosa* serotype in a sample. In still another embodiment of the invention, the kit includes primers which are capable of amplifying a nucleic acid molecule of the invention or a predetermined oligonucleotide fragment thereof, all the reagents required to produce the amplified nucleic acid molecule or predetermined fragment thereof in the polymerase chain reaction, and means for assaying the amplified sequences.

The methods and kits of the present invention have many practical applications. For example, the methods and kits of the present invention may be used to detect a *P. aeruginosa* serotype in any medical or veterinary sample suspected of containing *P .aeruginosa*. Samples which may be tested include bodily materials such as blood, urine, tissues and the like. Typically the sample is a clinical specimen from wound, burn and urinary tract infections. In addition to human samples, samples may be taken from mammals such as non-human primates, etc. Further, water and food samples and other environmental samples and industrial wastes may be tested.

Before testing a sample in accordance with the methods described herein, the sample may be concentrated using techniques known in the art, such as centrifugation and filtration. For the hybridization and/or PCR-based methods described herein, nucleic acids may be extracted from cell extracts of the test sample using techniques known in the art.

Substances that Affect O-antigen synthesis and assembly

A protein of the invention may also be used to assay for a substance which affects O-antigen synthesis or assembly in *P. aeruginosa* Accordingly, the invention provides a method for assaying for a substance that affects O-antigen synthesis or assembly in *P. aeruginosa* comprising mixing a protein of the invention with a test substance which is

suspected of affecting the expression or activity of the protein, and determining the effect of the substance by comparing to a control.

In an embodiment f the invention the protein is an enzyme, and a method is provided for assaying for a substance that affects O-antigen synthesis and assembly in *P. aeruginosa* comprising incubating a protein of the invention with a substrate of the protein, and a test substance which is suspected of affecting the activity of the protein, and determining the effect of the substance by comparing to a control.

In a preferred embodiment the protein is PsbM which has dehydrogenase activity. Representative substrates which may be used with PsbM in the assay are precursor sugars such as glucose. Dehydrogenase activity may be assayed using conventional methods.

Compositions and Methods of Treatment

The substances identified by the methods described herein, antisense nucleic acid molecules, and antibodies, may be used for modulating one or both of O-antigen synthesis and assembly in *P. aeruginosa* and accordingly may be used in the treatment of infections caused by *P.aeruginosa*. O-antigen is a virulence factor of *P. aeruginosa* and it is responsible for serum resistance. Therefore, substances which can target LPS biosynthesis in *P. aeruginosa* to change the organism into making "rough" LPS devoid of the long chain O-antigen (B-band) polymers will be useful in rendering the bacterium susceptible to attack by host defense mechanisms. The substances identified by the methods described herein, antisense nucelic acid molecules, and antibodies are preferably used to treat infections caused by *P. aeuginosa* serotypes 02, 05, 16, 18 and 20. The substances etc. are also preferably used to treat infections caused by *P. aeruginosa* serotypes 03 or 06 which are predominant clinical isolates. It will be appreciated that the substances may also be useful to treat infections caused by other members of the family Pseudomonadaceae (eg. P. cepacia and P. pseudomallei), and to treat other bacteria which produce O-antigen, (e.g. other gram negative bacteria such as *E. coli, S. enterica, Vibrio cholera, Yersinia entercolitica* and *Shigella flexneri*).

The substances identified using the methods described herein may be formulated into pharmaceutical compositions for adminstration to subjects in a biologically compatible form suitable for administration *in vivo*. By "biologically compatible form suitable for administration *in vivo*" is meant a form of the substance to be administered in which any toxic effects are outweighed by the therapeutic effects. The substances may be administered to living organisms including humans, and animals. Administration of a therapeutically active amount of the pharmaceutical compositions of the present invention is defined as an amount effective, at dosages and for periods of time necessary to achieve the desired result. For example, a therapeutically active amount of a substance may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of antibody to elicit a desired response in the individual. Dosage regima may

be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

The active substance may be administered in a convenient manner such as by injection (subcutaneous, intravenous, etc.), oral administration, inhalation, transdermal application, or rectal administration. Depending on the route of administration, the active substance may be coated in a material to protect the compound from the action of enzymes, acids and other natural conditions which may inactivate the compound.

The compositions described herein can be prepared by per se known methods for the preparation of pharmaceutically acceptable compositions which can be administered to subjects, such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985). On this basis, the compositions include, albeit not exclusively, solutions of the substances in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids.

The reagents suitable for applying the methods of the invention to identify substances that affect O-antigen synthesis and assembly in *P. aeruginosa* may be packaged into convenient kits providing the necessary materials packaged into suitabl containers. The kits may also include suitable supports useful in performing the methods of the invention.

The utility of the substances, antibodies, and compositions of the invention may be confirmed in experimental model systems.

The invention will be more fully understood by reference to the following examples. However, the examples are merely intended to illustrate embodiments of the invention and are not to be construed to limit the scope of the invention.

EXAMPLES

Materials and methods used in Examples 1 to 3 described herein include the following:

Bacterial strains and culture conditions

The bacterial strains used in this study are listed in Table 6. All bacterial strains were maintained on Tryptic Soy Agar (Difco Laboratories, Detroit, MI). P. Isolation Agar (PIA; Difco) was used for selection of transconjugants following mating experiments. Antibiotics used in selection media include: ampicillin at 100 µg/ml for *E. coli* and carbenicillin at 450 µg/ml for *P. aeruginosa*, tetracycline at 15 µg/ml for *E. coli* and 90

μg/ml for *P. aeruginosa* (250 μg/ml in PIA), gentamicin at 10 μg/ml for *E. coli* and 300 μg/ml for *P. aeruginosa*.

DNA procedures

Small-scale preparation of plasmid DNA was done utilizing the alkaline lysis method of Birnboim and Doly (1979). Large-scale preparations of plasmid DNA were obtained using the Qiagen midi plasmid kit (Qiagen Inc., Chatsworth, CA), according to procedures specified by the manufacturer. Whole genomic DNA was isolated from *P. aeruginosa* following the method of Goldberg and Ohman (1984). Restriction enzymes were purchased from GIBCO/BRL and Boehringer-Mannheim (Mannheim, Germany). T4 DNA ligase, T4 DNA polymerase and alkaline phosphatase were purchased from Boehringer-Mannheim. All enzymes were used following suppliers' recommendations. DNA was transformed into *E. coli* and *P. aeruginosa* by electroporation using a Bio-Rad electroporation unit (Bio-Rad Laboratories, Richmond, CA) and according to the protocols supplied by the manufacturer. Electrocompetent cells of *E. coli* and *P. aeruginosa* were prepared according to the methods of Binotto *et al.* (1991) and Farinha and Kropinski (1990), respectively. Recombinant plasmids were mobilized from *E. coli* DH5α to *P. aeruginosa* through triparental matings as described by Ruvkun and Ausubel (1981). Plasmids were also mobilized from *E. coli* SM10 to *P. aeruginosa* using the method of Simon *et al.* (1983). Genomic DNA was transferred to Zetaprobe membrane (Bio-Rad) by capillary transfer following the manufacturer's instructions. Southern hybridizations were done at 42°C for 18-24h with DNA previously labelled with dUTP conjugated to digoxigenin (DIG) (Boehringer-Mannheim). Labelling of DNA was done according to the manufacturer's recommendations. Hybridized DNA was detected using an anti-DIG polyclonal antibody conjugated to alkaline phosphatase and AMPPD (0.235 mM 3-(2'-Spiroadamantane)-4-methoxy-4(3''-phosphoryloxy)-phenyl-1,2-dioxetane) (Boehringer-Mannheim), followed by exposure to X-ray film (E. I. Du Pont de Nemours & Co., Wilmington, DE).

Tn*1000* mutagenesis of pFV.TK6

Tn*1000* mutagenesis of pFV.TK6 was performed as described previously (Lightfoot and Lam, 1993) using the method of de Lencastre *et al.* (1983).

DNA sequencing

DNA sequence analysis of the 1.9 kb insert of pFV.TK8 was performed by the MOBIX facility (McMaster University, Hamilton ON). The 1.9 kb *XhoI-HindIII* insert of pFV.TK8 was cloned into the sequencing vector pBluescript II KS and double-strand sequenced using a model 373A DNA sequencing unit (Applied Biosystems, Foster City, CA). Oligodeoxynucleotide primers for sequencing were synthesized on an Applied Biosystems model 391 DNA synthesizer and purified according to the manufacturers' instructions. The Taq DyeDeoxy™ Terminator Cycle Sequencing Kit (Applied Biosystems) was used for cycle

sequencing reactions which were carried out in an Ericomp (San Diego, CA) model TCX15 thermal cycler.

Sequence Analysis

The computer software programs Gene Runner for Windows (Hastings Software, New York, NY) and PCGENE (IntelliGenetics, Mountain View, CA) were used for nucleic acid sequence analysis, amino acid sequence analysis, and characterization of the predicted protein. DNA and protein database searches were performed using the NCBI BLAST network server (Altschul *et al.*, 1990; Gish and States, 1993).

Mutagenesis of the *rfc* gene of *P. aeruginosa* PAO1

In order to construct *P. aeruginosa rfc* chromosomal mutants a novel gene replacement vector, pEX100T (Schweizer and Hoang , 1995) was used. This vector, called pEX100T, contains the *sacB* gene of *B. subtilis* which imparts sucrose sensitivity on gram-negative organisms and allows for positive selection of true mutants from the more frequently occurring merodiploids. In the first step of this experiment, the 5.6 kb *Hin*dIII fragment of pFV.TK6 was blunt-ended using T4 DNA polymerase and subcloned into the *Sma*I site of pEX100T. An 875 bp Gm^R cassette from pUCGM (Schweizer, 1993) was then cloned into the single *Bam*HI site of the insert DNA. The resulting plasmid, pFV.TK9, was transformed into the mobilizer strain *E. coli* SM10 and then conjugally transferred into PAO1 (Simon *et al.*, 1983). After mating, cells were plated on PIA containing 300 µg/ml of Gm. Colonies that grew on the Gm-containing medium were picked and streaked on PIA containing 300 µg/ml Gm and 5% sucrose to identify isolates that had lost the vector-associated *sacB* gene, and thus had become resistant to sucrose. Southern blot analysis was performed to verify that gene replacement had occurred (Figure 24).

Preparation of LPS

LPS used in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western immunoblotting experiments was prepared according to the proteinase K digest method of Hitchcock and Brown (1983).

SDS-PAGE

The discontinuous SDS-PAGE procedure of Hancock and Carey (1979) utilizing 15% running gels was used. LPS separated by SDS-PAGE was visualized by silver-staining according to the method of Dubray and Bezard (1982).

Immunoblotting

The Western immunoblotting procedure of Burnette .981) was used with the following modifications. Nitrocellulose blots were blocked with 3% (w/v) skim milk followed by incubation with hybridoma culture supernatant containing either MAb MF15-4, specific for O5 LPS, or MAb N1F10, specific for A-band LPS. The blots were developed at room temperature, using goat anti-mouse $F(ab')_2$ fragment conjugated antibody

(Jackson Immunoresearch Laboratories, West Grove, PA) and a substrate consisting of 30 mg of Nitro Blue Tetrazolium and 15 mg of 5-bromo-4-chloro-3-indolyl phosphate toluidine (Sigma, St. Louis, MO) in 100 ml of 0.1 M bicarbonate buffer (pH 9.8).

EXAMPLE 1

Analysis of the LPS from mutants AK14O1 and rd7513. Strain AK14O1 has been previously shown to contain A-band LPS; its B-band LPS consists of complete core plus one O-repeat unit (SR phenotype) (Berry and Kropinski, 1986; Lam *et al.*, 1992). Strain rd7513 is a mutant of AK14O1 that has the SR phenotype but is no longer producing A-band LPS, due to a mutation in an A-band biosynthetic gene (Lightfoot and Lam, 1991). Strain rd7513 was used in this study described in the examples, in addition to AK14O1; but the majority of this investigation will focus on AK14O1.

Complementation of O-antigen expression in *P. aeruginosa* AK14O1. Mobilization of pFV100, which contains the O5 *rfb* gene cluster, into SR mutant AK14O1 resulted in production of O5 B-band LPS. These results suggest that an O-polymerase gene might be localized on the cloned DNA. Analysis of LPS isolated from PAO1 and AK14O1(pFV100) in both silver-stained SDS-PAGE gels and Western immunoblots, reacted with O5-specific MAb MF15-4, revealed that the two strains expressed similar high molecular weight LPS profiles (Figure 22 a, b). In order to localize the putative *rfc* gene on the 26 kb insert of pFV100, various subclones were made (Figure 23) and used in complementation studies with AK14O1. Plasmid pFV.TK2, which contains a 16.5 kb *Xba*I fragment from pFV100 was able to complement O5 O-antigen production after mobilization into AK14O1 (data not shown). Plasmids pFV.TK3, pFV.TK4, and pFV.TK5 were generated and mobilized into AK14O1, however none of the three plasmids was able to complement B-band synthesis in this mutant. Subsequently, pFV.TK6 which contains a 5.6 kb *Hin*dIII insert was made and was able to complement the SR phenotype of AK14O1 (data not shown).

Transposon Tn*1000* mutagenesis of pFV.TK6. Transposon mutagenesis using Tn*1000* was performed in order to more precisely define the region of insert DNA in pFV.TK6 responsible for complementation of O-antigen expression in AK14O1. pFV.TK6::Tn*1000* recombinants were mobilized into AK14O1 and then screened for the lack of expression of O-antigen using O5-specific MAb MF15-4. Plasmid DNA was isolated from colonies that did not react with MAb MF15-4, and subjected to restriction enzyme analysis to determine the location of the Tn*1000* insertion in pFV.TK6. Three Tn*1000* insertions in a 1.5 kb *Xho*I fragment were found to interrupt O-antigen expression in AK14O1 (Fig. 23). This 1.5 kb *Xho*I fragment was cloned into vector pUCP26 (pFV.TK7) and mobilized into AK14O1. In Western immunoblots of LPS from AK14O1(pFV.TK7) with MAb MF15-4 no reaction of this antibody with high molecular weight B-band LPS could be detected (data not shown). Therefore, the 1.5 kb *Xho*I insert in pFV.TK7 was unable to restore the O-polymerase

function in AK14O1. A 1.9 kb *Xho*I-*Hin*dIII fragment was then subcloned into pUCP26 and the resulting plasmid was designated pFV.TK8 (Figure 23). Mobilization of this recombinant plasmid into both SR mutants, AK14O1 and rd7513, resulted in restoration of O-antigen expression. Silver-stained SDS-PAGE gels and Western blots reacted with MAb MF15-4, showed that the AK14O1(pFV.TK8) transconjugants expressed levels of O5 B-band LPS comparable to that produced by the wild-type PAO1 (Figure 22).

Southern analysis using a 1.5 kb *Xho*I probe. The 1.5 kb *Xho*I insert of pFV.TK7, internal to the *rfc* coding region, was labelled with dUTP conjugated to digoxigenin and used to probe *Xho*I-digested chromosomal DNA from the twenty *P. aeruginosa* serotypes. The probe hybridized to a 1.5 kb fragment in serotypes O2, O5, O16, O18 and O20 (data not shown), suggesting that these serotypes may share a similar O-polymerase gene. These hybrization results are not surprising in that serotypes O2, O5, O16, and O20 share a similar O-repeat backbone structure (Knirel, 1990). Although the O-antigen structure of serotype O18 has not yet been determined, it exhibits cross-reactivity with polyclonal antisera raised against serotype O5 (data not shown), suggesting that it has an O-repeat unit structure similar to that of O5. In a recent study, Collins and Hackett (1991) found that a probe generated from the *rfc* gene of *S. enterica* (*typhimurium*) cross-hybridized to chromosomal DNA of *Salmonella* groups A, B, and D1 strains but not with strains of groups D2 or E2, suggesting that the former may share a common *rfc* gene. In addition, studies done by Nurminen and coworkers (1971) have shown that the O-polymerase enzymes of *Salmonella* groups B and D1 strains are able to polymerize O-repeat units of either serotype.

Generation of *P. aeruginosa* chromosomal *rfc*-mutants. In order to confirm that the insert DNA of pFV.TK8 codes for an O-polymerase gene, insertional mutagenesis was performed and the resulting plasmid used for homologous recombination with the PAO1 chromosome. In the first step, the 5.6 kb insert of plasmid pFV.TK6 was cloned into a novel gene replacement vector, pEX100T, (Schweizer and Hoang, 1995). pEX100T is a pUC19-based plasmid that does not replicate in *P. aeruginosa*; therefore, maintenance of plasmid DNA can only occur after homologous recombination into the chromosome. The 5.6 kb insert of pFV.TK6 was used for gene replacement instead of the 1.9 kb insert of pFV.TK8 to ensure that there was sufficient DNA for homologous recombination. The next step involved insertion of an 875 bp Gm^R cassette into a unique *Bam*HI site in the insert DNA (Figure 24b). This step generated a mutation in the *rfc* gene and provided a means of later selecting for colonies that had undergone homologous recombination. Because the vector, pEX100T, contains the *sacB* gene of *Bacillus subtilis* it renders Gram-negative organisms sensitive to sucrose. Streaking Gm^R recombinants on media containing 5% sucrose allowed separation of true recombinants from merodiploids, since merodiploids exhibit sucrose-sensitivity because of the presence of the vector-associated *sacB* gene. Of the eighty Gm^R colonies that were

is lated, twenty-four were found to be sucrose-resistant. Three of the twenty-f ur isolates were randomly chosen for further characterization and were designated OP5.2, OP5.3, and OP5.5. Southern blot analysis f chromosomal DNA from these three putative mutants was performed in order to confirm that gene replacement had occurred. The 1.5 kb *Xho*I fragment of pFV.TK8 was used to probe *Xho*I-digested chromosomal DNA isolated from the PAO1 wild-type strain as well as OP5.2. OP5.3, and OP5.5. In strains that had undergone gene replacement, *Xho*I digestion should yield a probe-hybridizable fragment of 2.4 kb instead of 1.5 kb because of the insertion of the 875 bp Gm^R cassette (Figure 24 a, b). Southern blot analysis of the three Gm^R, sucrose-resistant isolates revealed a probe-reactive fragment of 2.4 kb (Figure 24 c, lanes 2-4); whereas, the probe reacted with a 1.5 kb fragment of the PAO1 control DNA (Figure 24 c, lane 1), demonstrating that gene replacement had occurred in OP5.2, OP5.3, and OP5.5. Analysis of LPS from these three strains in silver-stained gels and Western immunoblots with O5-specific MAb MF15-4 demonstrated that they were not capable of producing long chain B-band O-antigen (Fig. 25a, b). Immunoblots reacted with A-band specific MAb N1F10 revealed that, like the SR mutant AK14O1, these three mutants were still producing A-band LPS (Figure 25c). Biosynthesis of A-band LPS therefore, appears to be unaffected by this chromosomal mutation. The relative mobility of the core-lipid A bands was also similar to that of the SR mutant AK14O1 (Figure 25a); therefore the LPS phenotype of the three *rfc* knockout mutants was identical to that of AK14O1. Mobilization of pFV.TK8 into OP5.2, OP5.3 and OP5.5 restored O-antigen expression in the three mutants (data not shown), indicating that the PAO1 chromosomal modification was the result of a direct mutation of the *rfc* gene and not caused by a secondary mutation.

Nucleotide sequence determination and analysis of *rfc*. The 1.9 kb *Xho*I-*Hin*dIII insert of pFV.TK8, containing the *rfc* coding region, was cloned into pBluescript and subjected to double-strand nucleotide sequence analysis. Examination of the nucleotide sequence (Figure 9; GenBank accession number U17294) revealed one open reading frame (ORF) that coded for a protein of 438 amino acids, with a predicted mass of 48.9 kDal. This ORF was designated ORF48.9.

Analysis of the *P. aeruginosa rfc* mol. % G + C content (44.8%; Table 6) revealed that it is significantly lower than that of the rest of the genome (67.2%; Palleroni, 1984). A low G + C content is a common feature of reported *rfc* genes (Collins and Hackett, 1991; Brown *et al.*, 1992; Klena and Schnaitman, 1993; Morona *et al.*, 1994) and has also been observed in all of the *rfb* clusters so far analyzed. The finding that the gene coding for the O-polymerase enzyme and the genes encoding the O-antigen repeat units have a compatible G + C content is not surprising since the specificity of the enzyme must relate to the structure of it substrate.

Homology searches of both the nucleotide and the amino acid sequences of the *P. aeruginosa rfc* gene were performed using EMBL/GenBank/PDB and Swiss-PROT (release 28.0) databases (Altschul *et al.*, 1990; Gish and States, 1993). Comparison of the *P. aeruginosa rfc* sequences with sequences reported for other prokaryotic genes revealed no significant homology, including with those reported for other *rfc* genes. Previous studies on the structure of *P. aeruginosa* O-antigens have revealed that their sugar compositions differ significantly from most other enterobacterial O-antigens (Knirel *et al.*, 1988). Neutral sugars, which are commonly found in enteric O-antigens, are only rarely found in O-antigens of *P. aeruginosa*. In addition, *P. aeruginosa* O-antigens are rich in amino sugars, many of which are substituted with acyl groups, a phenomenon rarely found in natural carbohydrates. Given the unique sugar composition of *P. aeruginosa* O-antigens, and the finding by Morona *et al.* (1994) that the *S. flexneri* Rfc protein showed no homology with other enteric Rfc proteins, it is not surprising that the *P. aeruginosa* Rfc protein exhibited no sequence homology with those of other enteric organisms.

The *P. aeruginosa rfc* gene product does, however, have several features in common with other reported Rfc proteins, including the fact that it is very hydrophobic. The mean hydropathic index of the *P. aeruginosa* Rfc is 0.8 while those of other enteric organisms have been reported to range from 0.65 - 1.08 (Table 7). Examination of the hydropathy profile of this protein and analysis of the amino acid sequence, using the software program PCGENE, revealed that it is an integral membrane protein with 11 putative membrane-spanning domains (Klein *et al.*, 1985). The Rfc proteins of *S. enterica* (*typhimurium*) and *S. enterica* (*muenchen*) are reported to have 11 membrane-spanning domains, while that of *S. flexneri* is reported to have 13 (Morona *et al.*, 1994); therefore, structural similarities appear to exist among the Rfc proteins of these four organisms.

Codon usage and amino acid composition analysis. When the codon usage and amino acid composition of the *P. aeruginosa* Rfc protein was compared with that reported for *S. enterica* (*typhimurium*), *S. enterica* (*muenchen*), and *Shigella flexneri* Rfc proteins (Collins and Hackett, 1991; Brown *et al.*, 1992; Morona *et al.*, 1994), significant similarities were found between them (data not shown). Rfc proteins have been reported to contain a high content of three amino acids, namely, leucine, isoleucine, and phenylalanine (Morona *et al.*, 1994). These three amino acids account for 27, 30, and 37 % of the total amino acids of the Rfc proteins of *S. enterica* (*typhimurium*), *S. enterica* (*muenchen*), and *Shigella flexneri*, respectively (Morona *et al.*, 1994). In the Rfc protein of *P. aeruginosa*, these amino acids represent 30% of the total amino acid composition.

In summary, the present inventors have isolated an *rfc* gene in *P. aeruginosa* O5 encoding an O-polymerase enzyme. Using a gene-replacement system, *P. aeruginosa rfc*-chromosomal mutants were generated which expressed the typical sr lps

phenotype. The *P. aeruginosa* Rfc is similar to other reported Rfc proteins in that it is very hydrophobic, containing 11 membrane-spanning domains; the Rfc coding region has a lower mol. % G + C than the *P. aeruginosa* chromosomal average; and it has a similar amino acid composition and codon usage to that reported for other Rfc proteins.

EXAMPLE 2

Isolation of a *rol* gene in *P. aeruginosa* 05 (PA01) Encoding a Protein which Regulates O-antigen Chain Length

The *P. aeruginosa* serotype 05 (PA01) *rol* gene (regulator of O-chain length) was cloned from a genomic DNA cosmid library. An open reading frame (ORF) of 1046 bp, encoding a 39.3 kDa protein, was identified. The characterization of the function of Rol was facilitated by the generation of knockout mutants.

The DNA sequence of a subclone of pFV100, pFV161 (Figure 26), was found to have homology to the *rol* genes from a number of members of the family *Enterobacteriaceae*. However, only the 3' end of the putative *rol* gene was present on pFV161. A cosmid library of *P. aeruginosa* (PA01) genomic DNA was screened using a digoxigenin-labeled probe from pFV161 to identify an overlapping cosmid (pFV400) containing the complete *rol* gene. Southern blot analysis of DNA from pFV400, digested with a number of different restriction enzymes, was performed. The pFV161 probe hybridized to an approximately 2.3 kb *Hin*dIII fragment of pFV400. Assuming the *rol* gene of *P. aeruginosa* serotype 05 (PA01) was similar in size (approx. 1 kb) to members of the family *Enterobacteriaceae* (Morona *et al.*, 1995), this fragment would be sufficient to contain the entire putative *rol* gene. This 2.3 kb *Hin*dIII fragment was subcloned into the vector pBluescript II SK (PDI Biosciences, Aurora, Ontario, Canada) and named pFV401 (Figure 26).

Nucleotide sequencing of the 2.3 kb *Hin*dIII insert was performed using dye terminator cycle sequencing (GenAlyTiC sequencing facility, University of Guelph), and an open reading frame (ORF) that coded for a protein of 348 amino acids, with a predicted mass of 39.3 kDA, was identified (GenBank accession #U50397). Homology searches using the GenBank database through the NCBI Blast network server were performed (Altschul *et al.*, 1990; Gish and States, 1993). Both the nucleotide and the deduced amino acid sequences of the putative *P. aeruginosa rol* gene showed approximately 33-35% amino acid homology between the putative Rol protein and the Rol proteins of *Salmonella enterica* serovar typhimurium, *Escherichia coli*, and *Shigella flexneri* (Morona *et al.*, 1995) (Table 5).

To confirm that the insert DNA of pFV401 codes for a Rol protein, insertional mutagenesis was performed and the resulting plasmid construct used for homologous recombination with the PA01 chromosome. Briefly, the 2.3 kb insert of pFV401 was cloned into a novel gene-replacement vector, pEX100T (Schweizer and Hoang, 1995),

that does not replicate in *P. aeruginosa*. pEX100T also contains the *sacB* gene of *B. subtills* which imparts sucrose sensitivity on Gram-negative organisms and allows for positive selection of true mutants from the more frequently occurring merodiploids. Next, an 875 bp gentamicin-resistance (GM^R) cassette from pUCGM (Schweizer, 1993) was inserted into a unique *Xho*I site in the insert DNA. The resulting plasmid (pFV401TG) was transformed into the mobilizer strain *E. coli* SM10 and then conjugally transferred into PA01 (Simon *et al.*, 1983). After mating, cells were plated on *P.* isolation agar (PIA; Difco Laboratories, Detroit, Mich.) containing 300μg ml^{-1} gentamicin (Sigma Chemical Co., St. Louis, Mo.) and 5% sucrose. This selective medium allows the identification of isolates that have undergone homologous recombination and lost the vector-associated *sacB* gene thus, becoming resistant to sucrose. Southern blot analysis with both wild-type *rol* gene and Gm^R cassette probes was used to confirm the insertional mutation. The wild-type control and the mutants showed probe reactive fragments of 2.3 kb and 3.1 kb respectively (Fig. 27).

The LPS of the mutants was prepared according to the proteinase K digest method of Hitchcock and Brown (1983). The LPS was analyzed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western immunoblots according to the methods described previously (de Kievit *et al.*, 1995). When compared with the wild-type strain, the mutant LPS showed a marked alteration in the O-antigen ladder-like banding pattern, in which there was a decrease in high molecular weight bands and an increase in visible low molecular weight bands. This change corresponds to a loss of bimodal distribution in O-antigen length (Fig. 28).

A T7 expression system (Tabor and Richardson, 1985) was used for expression of the Rol protein. A unique protein band with an apparent molecular mass of 39 kDa was observed. This expressed polypeptide corresponded well to the predicted mass of 39.3 kDa. This band was not observed in the vector-only control (Fig. 29).

In conclusion, a *rol* gene was isolated in *P. aeruginosa* 05 (PA01) encoding a protein which regulates O-antigen chain length. Using a gene-replacement system, *P. aeruginosa rol*::Gm^R knockout mutants were generated which express LPS with unregulated O-antigen chain length. Thus, the *P. aeruginosa* 05 (PA01) Rol protein has both sequence and functional homology to other reported Rol proteins. This also confirms that the pathway for *P. aeruginosa* B-band LPS biosynthesis is Rfc-dependent. The function of Rol is often associated with the Rfc protein, an O-polymerase (Whitfield, 1995, Kievit *et al.*, 1995).

EXAMPLE 3

Sequencing of the *psb* gene cluster.

The isolation of a cosmid clone, pFV100, containing the *psb* gene cluster of *P. aeruginosa* O5 identified in accordance with the present invention, was previously

described (Lightfoot and Lam, 1993). Several subclones of pFV100 containing the *psb* genes were constructed. The sequencing and characterization f two of these cl nes (pFV111 and pFV110), containing the *rfc* and *psbL* (*rfbA*) genes respectively, has previously been described (de Kievit et al., 1995; Dasgupta and Lam, 1995). Sequencing of the remainder f the pFV100 insert was undertaken in order to identify all the genes required for synthesis of the O5 O-antigen.

Sequencing of the entire insert of pFV100, a total of 24416 bp, revealed a large number of open reading frames (ORFs) on both strands. ORFs which were reading in the same direction as *rfc* and *psbL* and which had homology either to any previously identified polysaccharide or antibiotic biosynthetic genes or to highly conserved bacterial genes were characterized further. A total of 21 ORFs which could be involved in synthesis of the O5 O-antigen were identified (Table 1). These genes were designated *psbA* through *psbN* in the 5' to 3' direction, with the exceptions of *rol* and *rfc*, which were named according to convention. A further 4 ORFs with high homology to other bacterial genes or insertion sequences but which are not thought to be involved with LPS synthesis were identified (*hisH*, *hisF*, *uvrB*, IS*407*; Table 1).

Distribution of the *psb* genes among the 20 serotypes of *P. aeruginosa* and localization of the O5-specific region.

Southern blot analysis of the 20 serotypes of *P. aeruginosa* using various *psb* genes as probes revealed an interesting dichotomy. All of the probes tested which were 5' to the IS*407* element hybridized only with chromosomal DNA from serotypes O2, O5, O16, O18 and O20 (Table 1). As stated above, these five serotypes have biochemically and structurally similar O-antigens (Figure 1). Although the O-antigens of serotypes O2, O5, O16, O18, and O20 are serologically distinct and have been shown to have clear biochemical differences, none of the *psb* genes tested hybridized only to serotype O5 chromosomal DNA at high stringency.

In contrast with these findings, probes for DNA sequences 3' to the IS*407* element, and the IS*407* element itself, hybridized with the chromosomal DNA from all 20 serotypes of *P. aeruginosa* (Table 1). These results show that the insertion sequence is the junction between the portion of the *psb* cluster specific for O5 and related serotypes (hereinafter referred to as the O5-specific region, or sometimes as the Group I genes) and the non-specific chromosomal DNA. Therefore, *psbL* appears to be the last gene of the O5-specific region. Despite the fact that the DNA 3' of the insertion element is not O5-specific, this region is thought to contain at least two ORFs (*psbM* and *psbN* or sometimes referred to as the Group II genes) which may be involved in O5 LPS biosynthesis (see below).

A 1.2 kb probe from the extreme 5' end of the insert of pFV100 hybridized only to the five related serotypes, indicating that the 5' end of the O5-specific region had not been cloned. This probe was used to isolate an overlapping cosmid, pFV400. Various subclones of pFV400 were constructed to localize the 5' end of the O5-specific region to within a 1.3 kb *SstI-XhoI* fragment located 1.7 kb upstream of the 5' end of pFV100. Preliminary sequence analysis of this upstream region revealed no additional ORFs thought to be involved with LPS synthesis. Also, no insertion sequences could be found in this region of DNA. Localization of the 5' end of the O5-specific region to the 1.3 kb *SstI-XhoI* fragment means the total amount of DNA which is specific to O5 and related serotypes is approximately 20 kb.

The composition and chromosomal milieu of the O5 *psb* cluster.

The %G+C of the *P. aeruginosa* chromosome has been determined by various methods to be approximately 65-67% (Palleroni, 1984; West and Iglewski, 19XX). The %G+C content of the *P. aeruginosa* O5 *psb* cluster within the O5-specific region averages 51.1% overall, with individual genes ranging from a low of 44.5% (*psbG*) to a high of 56.8% (*psbK*) (Table 1). These results are consistent with those seen for other *rfb* genes, averaging at least 10% below the chromosomal background, and this is thought to be reflective either of origin in a low %G+C background (Reeves, 1993) or of possible regulatory constraints (Collins and Hackett, 1991; Morona et al., 1994a). The %G+C content of the *psbM* and *psbN* genes, which fall outside the O5-specific region, averages 62.6 %.

Sequence analysis of pFV100/pFV400 revealed no homology to *gnd* (encoding 6-phosphogluconate dehydrogenase) in the regions flanking the LPS genes. However, *P. aeruginosa* has been shown to convert glucose-6-phosphate to 6-phosphogluconate as part of the Entner-Doudoroff pathway, suggesting a homologue of the *gnd* gene is located elsewhere on the chromosome. The location of the *P. aeruginosa his* operon is not known, but the few *his* auxotrophic lesions that have been mapped on the chromosome of serotype O5 (strain PAO1) are several minutes from the A- and B-band LPS clusters (Lightfoot and Lam, 1993; Hollaway et al., 1994). Interestingly, two *his* genes (*hisH* and *hisF*) were found in the middle of the *psb* cluster, within the O5-specific region (see below). Because these genes fail to hybridize with all twenty serotypes of *P. aeruginosa* at high stringency, it is likely they are not native *P. his* genes, but were acquired along with the *psb* genes in a horizontal transfer event.

Homology searches of the Genbank databases with each of the ORFs in the *psb* cluster were performed. Assignment of putative function for the products of the ORFs was made based on homology of the encoded proteins to those previously described. Because the O-antigen of *P. aeruginosa* O5 contains two similar 2,3-diacetaminido-

mannuronic acid residues, it is anticipated that both residues share a comm n biosynthetic pathway.

The 5' end of th pFV100 insert contains a partial *rol* gene.

The partial open reading frame at the 5' end of the insert of pFV100 was found to have low homology at the amino acid level (34-37%) with the Rol proteins of *Escherichia coli* (Batchelor et al., 1992; Bastin et al., 1993), *Salmonella enterica* sv Typhimurium (Batchelor et al., 1992; Bastin et al., 1993), and *Shigella flexneri* (Morona et al., 1994b). Only 479 bp of *rol*-homologous DNA (encoding 159 amino acids) were present from the *Xho*I cloning site of pFV100. This sequence represented approximately the 3' half of the putative *rol* gene, based on the sizes of previously described *rol* genes. Using the partial gene as a probe, the entire *rol* gene has been cloned from an overlapping cosmid, pFV400, and its function confirmed by mutational analysis (Example 2). In other Rfc-dependent LPS gene clusters, the *rol* gene is positioned near or at the end of the cluster. These results, along with the large number of ORFs already identified on pFV100 suggested that most, if not all, of the genes required for O5 O-antigen biosynthesis are present on this cosmid.

***psbA*.**

There is a distance of 807 bases between the *rol* gene and the first adjacent gene, *psbA*. Although *P. aeruginosa* promoters are not well defined, there are similarities with *E. coli* promoters (Harley and Reynolds, 1987; Deretic et al., 1989). There is a possible σ^{70} -like promoter sequence and a putative ribosomal binding site (RBS) located 93 bp and 7 bp, respectively, upstream of the start of *psbA* (Figure 31). PsbA has homology (summarized in Table 2) to EpsD, thought to be a dehydrogenase required f r synthesis of exopolysaccharide in *Burkholderia solanaceraeum* (Huang and Schell, 1995); to VipA, involved in synthesis of the Vi antigen in *S. enterica* sv Typhi (Hashimoto et al., 1993); and to RffD, a UDP-*N*-acetyl-D-mannosaminuronic acid dehydrogenase involved in synthesis of Enterobacterial Common Antigen (ECA) in *E. coli* (Meier-Dieter et al., 1992). ECA is an exopolysaccharide common to most enterics that can be linked to lipid A-core in rough strains. It is composed of *N*-acetyl-D-glucosamine (GlcNAc), *N*-acetyl-D-mannosaminuronic acid (ManNAcA), and 4-acetamido-4, 6-dideoxy-D-galactose (Fuc4NAc).

PsbA also has homology with CapL, involved in type 1 capsular polysaccharide production in *Staphylococcus aureus* (Lin et al., 1994). The type 1 capsule is composed of taurine, 2-acetamido-2-deoxy-fucose (Fuc2NAc) and 2-acetamido-2-D-galacturonic acid (Gal2NAcA). The sugar composition of both ECA and type 1 capsule are similar to the *P. aeruginosa* O5 O-antigen. PsbA also has a low level of homology with ORF7 of the Vi antigen region of *E. coli/Citrobacter freundii* (accession #Z21706), and

several GDP-mannose and UDP-glucose dehydrogenases, including AlgD of *P. aeruginosa* (Deretic et al., 1987). AlgD is a GDP-mannose dehydrogenase required for alginate synthesis. These homologies suggest that PsbA functions as a dehydrogenase involved in the biosynthesis of the mannuronic acid residues, possibly converting UDP-*N*-acetyl-D-mannosamine into UDP-*N*-acetyl-D-mannosaminuronic acid. A large number of dehydrogenases including PsbA (as well as PsbK and PsbM, below) contain a consensus nicotinamide adenosine dinucleotide (NAD)-binding domain, thought to be important for activity (Figure 33). An alignment of the amino acid sequences of some PsbA-like proteins is shown in Figure 34.

psbB.

The *psbB* gene start is 74 bases from the termination codon of *psbA*, but no separate promoter sequence for *psbB* could be detected. A putative RBS is located 6 bp from the initiation codon for *psbB* and the second codon is AAA, the preferred second codon in *E. coli* (Gold and Stormo, 1987; Figure 32). The *psbB* gene product is possibly an oxido-reductase, dehydratase, or dehydrogenase. It is 28.2% homologous to the LmbZ protein of *Streptomyces lincolnesis* required for lincomycin production (Peschke et al., 1995), and also has homology with the *pur10* gene product of *Streptomyces alboniger* required for puromycin production (Tercero et al., 1996). PsbB has 17% homology to the BplA protein from *B. pertussis* required for LPS production (Allen and Maskell, 1996) and even weaker homology to ORF334 and MocA from *Rhizobium meliloti* found in the operon for rhizopine catabolism (Rossbach et al., 1994). In *B. pertussis*, the BplA protein is thought to catalyze the final step in the biosynthesis of UDP-diNAcManA from UDP-diNAcMan (Allen and Maskell, 1996).

Several of the *psb* genes were found to have high homology with *bpl* genes, suggesting a common ancestry. *B. pertussis* has semi-rough LPS, with only one O-antigen unit attached to the core oligosaccharide. The composition of the *B. pertussis* O-antigen unit is *N*-acetylglucosamine (GlcNAc), 2,3-dideoxy-2,3-*N*-acetylmannosaminuronic acid (2,3-diNAcManA), and *N*-acetyl-*N*-methyl fucosamine (FucNAcMe) (Allen and Maskell, 1996). These sugars are similar to those comprising ECA, *S. aureus* type 1 capsule, and the *P. aeruginosa* O5 O-antigen. The amino acid homology between PsbB and BplA as well as the similarties in O-antigen unit composition suggest that PsbB could have a homologous function to that of BplA. Unlike the other putative dehydrogenases encoded in the *psb* cluster, PsbB does not contain a consensus NAD-binding domain.

psbC.

The start of *psbC* overlaps significantly (343 bases) with the stop of *psbB*, and *psbC* could encode a large protein of 85.3 kDa (766 amino acids). Careful scrutiny of the DNA sequencing results confirmed no sequencing errors were present. Protein

expression will determine whether this entire large ORF is translated. The large size of this protein may indicate it resulted from a fusion event. There is a weak potential RBS upstream of the AUG c don of *psbC* (Figure 32).

The carboxy-terminal portion of PsbC has homology with a hypothetical protein (HI0392) derived from the *Haemophilus influenzae* genome sequence (Fleischmann et al., 1995). HI0392 is a 245 amino acid protein of unknown function, with several hydrophobic domains, and is thought to be an integral membrane protein. There is homology between PsbC and the macrolide 3-O-acyltransferase *acyA* gene from the *Streptomyces thermotolerans* carbomycin biosynthetic cluster (Arisawa et al., 1995). PsbC also has weak homology with ExoZ of *R. meliloti*, involved in succinoglycan production (Buendia et al., 1991), and with NodX of *R. leguminosarum*, involved in nodulation (Davis et al., 1988). ExoZ is a 317 amino acid protein, also with multiple hydrophobic domains, while NodX is a 367 amino acid protein thought to be located in the cytoplasmic membrane. ExoZ and NodX genes are both putative 3-O-acyltransferases. A summary of the homologies between the above proteins is shown in Table 2. The similarities indicate PsbC, particularly the carboxy terminal portion, may have 3-O-acyltransferase activity, and could be involved in acetylation of the mannuronic acid residues in the O5 O-antigen.

psbD.

The *psbD* gene appears to be translationally coupled with the *psbC* gene, since its start codon overlaps the stop codon of *psbC*. A potential RBS is located 9 bp upstream of the *psbD* AUG codon (Figure 32). The product of the *psbD* gene is most homologous with the product of the *bplB* gene in the *B. pertussis* LPS biosynthetic cluster (Allen and Maskell, 1996). PsbD and BplB appear to bc O-acetyl transferases, and have some homology to serine O-acetyl transferases (CysE) from a variety of bacteria, including *Buchnera aphidicola* (Lai and Baumann, 1992), *Bacillus stearothermophilus* (Gagnon et al., 1994), *B. subtilis* (Ogasawata et al., 1994), *E. coli* (Denk and Bock, 1987), *S. enterica* sv Typhimurium (accession #P29847), *H. influenzae* (Fleischmann et al., 1995), and the plant *Arabidopsis thaliana* (Bogdanova et al., 1995) (Table 2, Figure 35). As with PsbC, PsbD is probably involved in the acetylation of the mannuronic acid residues comprising two-thirds of the O5 repeat unit. While *bplA* and *bplB* are contiguous on the *B. pertussis* chromosome, the *psb* homologues, *psbB* and *psbD* respectively, are separated by the large *psbC* gene.

psbE.

psbE has high homology with a *B. pertussis* LPS biosynthetic gene, *bplC*. *psbD* and *psbE* are adjacent to one another in the *psb* cluster, as are *bplB* and *bplC* in the *bpl* cluster (Allen and Maskell, 1996). However, they do not appear to be translationally coupled, since there are 86 bases between the end of *psbD* and the start of *psbE*. While there is a potential RBS 9 bp before the *psbE* start (Figure 32), it is not known

whether this gene can be transcribed from a promoter internal to the *psbD* gene. There are some sequences with weak homology to the *E. coli* consensus promoter sequence in that area.

Also homologous to PsbE are DegT, from *B. subtilis* (Takagi et al., 1990), *Saccharopolyspora erythraea* ErbS (ERYC1) involved in erythromycin synthesis (Dhillon et al., 1989), DnrJ from *Streptomyces peucetius* required for daunorubicin biosynthesis (Stutzman et al., 1992) and SpsC from *B. subtilis* involved in spore coat polysaccharide biosynthesis (Glaser et al., 1993) (summarized in Table 2). There is also weak homology between PsbE and both MosB for rhizopine synthesis in *R. meliloti* (Murphy et al., 1993) and YifI, a hypothetical protein in the *rffE/rffT* intragenic region of *E. coli* (Daniels et al., 1992). The proteins DegT/DnrJ/ERYC1/SpsC form a family of proteins formerly thought to form the DNA-binding component of sensory-transduction two-component regulatory systems. More recently, however, their function is suggested to be in the biosynthesis of 2,3-, 2,4-, and 2,6-dideoxy sugars such as the 2,3-dideoxy mannuronic acid produced by *P. aeruginosa* O5 (Thorsen et al., 1993). An alignment of the amino acid sequences of the PsbE-like proteins is shown in Figure 36.

The O-antigen polymerase, *rfc*.

The *rfc* gene starts 254 bases downstream of the end of the *psbE* gene. This gene was cloned, sequenced and characterized as described in Example 1. Knockout mutations generated by insertion of a gentamicin cassette into *rfc* were used to confirm this gene encoded the O-antigen polymerase. Gentamicin-resistant mutants were shown to have the semi-rough phenotype (See Example 1) characteristic of an *rfc* mutant (Mäkelä and Stocker, 1984).

***psbF*.**

The *psbF* gene appears to be translationally coupled with the *rfc* gene since they have an overlapping stop and start. There is a RBS sequence 8 bp upstream of the initiation codon of *psbF*. It is most homologous to the ExoT protein of *R. meliloti* (Glucksmann et al., 1993), which is thought to be involved in succinoglycan transport. There is also a small amount of homology to FeuC of *B. subtilis*, part of its iron uptake system (Quirk et al., 1994). PsbF is the most hydrophobic protein encoded by the *psb* cluster (Table 1) and has 9-10 membrane-spanning domains. This secondary structure is remniscent of that of RfbX, the putative flippase found in Rfc-dependent O-antigen clusters (Figure 37) (Schnaitman and Klena, 1993). Mutations in RfbX have been found to be unstable and deleterious to the host strain (Schnaitman and Klena, 1993). Recently Liu et al. (1996) confirmed that RfbX (Wzx) mutants accumulate one O-antigen unit on undecaprenol on the inside of the cytoplasmic membrane. PsbF knockout mutants generated by insertion of a gentamicin resistance cassette into *psbF* are both A and B-band minus (Figure 48). PsbF may be the *P. aeruginosa* O5 equivalent of RfbX.

The *hisH* and *hisF* genes.

The histidine operon, containing genes required for the biosynthesis of the amino acid histidine, has previously been shown to lie adjacent to the *rfb* clusters of several enteric species (reviewed in Schnaitman and Klena, 1993). Comparison of the chromosomal map locations of the *P. aeruginosa* O5 A- and B-band LPS clusters with those of known PAO1 *his* mutations showed there were no *his* genes located adjacent to either the *psa* (11-13 min) or *psb* (37 min) clusters (Lightfoot and Lam, 1993; Holloway et al., 1994). Therefore, the identification of two genes with high homology to the genes *hisF* and *hisH* of various bacterial species in the middle of the *psb* cluster was unexpected. The *hisH* and *hisF* genes are located between the *psbF* and *psbG* genes (Figure 1), and transcribed in the same direction. The direction of transcription of the *his* genes in previously characterized *rfb* clusters is opposite to that of the *rfb* genes (Ames and Hartman, 1974; Macpherson et al., 1994).

While the deduced amino acid sequence of *hisF* appears to give a complete open reading frame (from bases 10387 to 11142), the sequence of *hisH* appears to be lacking an AUG initiation codon at the location predicted for the start of the protein based on amino acid homology. However, there are potential starts at three GUG codons located 51, 72, and 132 bp upstream of the first AUG, located at base 9830. The size of the protein corresponding to the product of *hisH* is approximately 21 kDa, indicating it is probably translated from either of these putative starts. Only the GUG codon at 9777 is preceded by a good RBS (Figure 32); none of the other potential start codons have consensus RBS sites. N-terminal analysis of the HisH product will confirm the translational start.

Protein expression analysis of this region shows the products of these genes are expressed *in vitro* in both orientations, indicating there is a promoter region preceding the *his* genes that can be recognized by *E. coli*. Analysis of the sequence upstream of the putative start sites of *hisH* shows there is a potential promoter sequence with partial homology to the *E. coli* consensus -35 and -10 regions (Figure 31). This homology is within the range seen in previously reported *P. aeruginosa* promoter sequences that can function in *E. coli* (Deretic et al., 1989; Ronald et al., 1992).

In *K. pneumoniae*, the products of the *hisH* and *hisF* genes have been shown to form a heterodimeric enzyme complex required for the conversion of N'-[(5'phosphoribulosyl)-formimino]-5-aminoimidazole-4-carboxamide-ribonucleotide (5'-PRFAR) to imidazole glycerol-phosphate (IGP) and 5'-phosphoribosyl-4-carboxamide-5-aminoimidazole (ZMP) (Rieder et al., 1994). Although the products of the *hisH* and *hisF* genes have been shown to function together, the *hisH* and *hisF* genes themselves are separated by a third gene, *hisA* (Alifano et al., 1996). The *hisA* and *hisH* genes are highly

related and are thought to have arisen through gene duplication. The gene order of *hisHAF* has been found in all bacterial species characterized to date (Alifano et al., 1996).

Comparison of the amino acid sequence homologies of various HisF and HisH proteins (Tables 3 and 4) showed that the *P. aeruginosa psb* HisF and HisH proteins are not closely related to any of the HisF/HisH proteins characterized thus far. Comparisons of *P. aeruginosa psb* HisF with the other HisF proteins shown in Table 6 shows that it is the most distantly related protein of the group analyzed, at approximately 50% homology.

psbG.

There is a distance of 138 bp between *hisF* and *psbG*, and a putative promoter is identified in this region (Figure 31). A RBS is identified 4 bp from a putative GUG start and 7 bp from the adjacent AUG start codon (Figure 32). The optimum spacing of a RBS from the initiation site is 8 ±2 bp, suggesting the AUG codon is likely to be the start. PsbG has limited homology to ORF2 (11.2%) of *Vibrio cholerae* O-antigen (Comstock et al., 1996), and less homology with NfrB of *H. influenzae*, a formate-dependent nitrate reductase (Fleischmann et al., 1993), and Pfk, a phosphofructokinase of the Gram positive bacterium, *Lactococcus lactis* (Xiao and Moore, 1993). Interestingly, the homology is associated with NfrB centres around the metal binding recognition site CXXCH, of which there are five in NfrB and one in PsbG (amino acids 24-28).

Insertion of a gentamicin cassette into *psbG* results in B-band deficient mutants of PAO1, suggesting a role for it in O-antigen biosynthesis.

psbH.

There are 15 bp between *psbG* and *psbH*, however, no RBS can be detected upstream of the *psbH* start codon. The third codon is AAA (Figure 32). PsbH demonstrates low homology with CapM (14.2%) of *S. aureus* (Lin et al., 1994), involved in the synthesis of N-acetogalactosamino uronic acid. PsbH also has homology with a number of glycosyl transferases, including IcsA (17.1%) (accession #U39810) and RfaK (13%) (accession #U35713) of *Neisseria meningitidis*, RfbF (11.3%) of *Klebsiella pneumoniae* (Keenleyside and Whitfield, 1994). There is also a low level of homology with RfpB of *Shigella dysenteriae* (Göhmann et al., 1994), and BplH and BplE of *B. pertussis* (Allen and Maskell, 1996). These enzymes are likely to belong to a family of transferases involved in the addition of a similar sugar to the growing O-antigen unit.

RfpB, RfaK, and RfbF are glucosyl- or galactosyl transferases and it is likely that CapM is the transferase involved in the addition of N-acetogalactosaminouronic acid. This suggests that PsbH is one of the two ManA transferases.

PsbH also has very limited homol gy to the DnaK proteins of *R. meliloti* (Falah and Gupta, 1994) and *Agrobacterium tumefaciens* (Segal and Ron, 1995). However, the homology is concentrated around the central region of PsbH. DnaK is a chaperonin, and is thought to have a role in gene regulation. Homology around the functional domain of DnaK may suggest a role for *psbH*/PsbH in regulation of the *psb* cluster.

psbI.

The start codon of *psbI* overlaps the stop codon of *psbH*. A putative RBS is situated 6 bp upstream of the AUG start and the second codon is AAA (Figure 32). PsbI demonstrates strong homology with BplD of *B. pertussis* (Allen and Maskell, 1996) (Table 2). BplD is purported to initiate the first step in the biosynthesis of 2,3-diNAcManA. PsbI also demonstrates moderate homology to NfrC and ORF o389 (RffD) of *E. coli* (Daniels et al., 1992), EpsC of *Burkholderia solanacearum* (Huang and Schell, 1995), YvyH of *B. subtilis* (Soldo et al., 1993) and RfbC of *S. enterica* sv Borreze (Keenleyside and Whitfield, 1995). EpsC is thought to be involved in the biosynthesis of *N*-acetylgalactosaminuronic acid, and RfbC is thought to be UDP-*N*-acetylglucosamine 2-epimerase. Alignment of PsbI and related proteins is shown in Figure 10. Based on these homologies, it is likely that PsbI converts UDP-*N*-acetylglucosamine to UDP-*N*-acetylmannosamine as the first step in the biosynthesis of mannuronic acid. Interestingly, the genes encoding the remaining enzymes in this pathway are located upstream and somewhat removed from the *psbI* gene (*psbABDE*).

psbJ.

The distance between *psbI* and *psbJ* is 17 bp. A putative RBS is present immediately following the stop codon of *psbI*, 13 bp from the AUG start codon of *psbJ* (Figure 4). PsbJ demonstrates reasonable homology to BplE (52.6%) of *B. pertussis*, a glycosyl transferase thought to attach either 2,3-diNAcManA or FucNAcMe to the O-unit (Allen and Maskell, 1996) (Table 2). TrsE of *Yersinia enterocolitica* also has homology to PsbJ (Skurnik et al., 1995), and is thought to be one of the galactosyl- or mannosyl transferases. An alignment of PsbJ and PsbJ-like proteins is shown in Figure 39. As BplE also has limited homology with PsbH, it is likely that both PsbH and PsbJ are the transferases involved in the addition of the two mannuronic acid residues to the B-band O-antigen unit. PsbJ has two putative membrane-spanning domains at the N-terminus, and may be anchored in the cytoplasmic membrane.

psbK.

The start codon of *psbK* overlaps the stop codon of *psbJ*, and the second codon is AAA (Figure 32). PsbK demonstrates homology to a series of glucose dehydratases, including StrP of *Streptomyces glauciens* involved in streptomycin biosynthesis (accession

number 629223), ExoB of *R. meliloti* (Buendia et al., 1991), ORF o355 (incorrectly assigned RffE) of *E. coli* (Daniels et al, 1992, Macpherson et al., 1994), GraE of *Streptomyces violaceoruben* (Bechtold et al., 1995) and RfbB of a number of organisms including *N. meningitidis* (Hamerschmidt et al., 1994) and *E. coli* (Marolda and Valvano, 1995). Alignment of these proteins show the presence of an NAD-binding domain (GXXGXXG) near the N-terminal end (Figure 5; Macpherson et al., 1994). RfbB and o355 are known to be involved in the biosynthesis of FucNAc (Meier-Dieter et al., 1992). Based on these homologies, PsbK is thought to be dTDP-D-glucose 4,6-dehydratase, required as the second step in the biosynthesis of FucNAc.

psbL.

There are 59 bp between the end of *psbK* and the start of *psbL* but no RBS could be detected in the region preceding the double start codons (Figure 32. Identification of the *psbL(rfbA)* gene has previously been reported (Dasgupta and Lam, 1995). Further characterization of PsbL suggests it functions as a transferase, and is thought to initiate O-antigen unit biosynthesis with the addition of FucNAc to undecaprenol, based on its homology to Rfe. The alignment of PsbL with TrsF from *Y. enterocolitica* (Skurnik et al., 1995) and Rfe from *E. coli* (Daniels et al., 1992) is shown in Figure 40. Rfe is the initial transferase involved in the biosynthesis of ECA and some O-antigens (Schnaitman and Klena, 1993; Macpherson et al., 1994), transferring GlcNAc to undecaprenol (Meier-Dieter et al., 1992). Because the first transferase in the biosynthesis of O-antigen interacts with undecaprenol, it would be expected to be a hydrophobic protein. PsbL is the most hydrophobic (hydropathy index of 0.84, Table 1) of the three putative transferases encoded in the *psb* cluster (PsbH, PsbJ, PsbL).

IS*407*$_{Pa}$.

Following the *psbL* gene is an insertion sequence with 61.5% nucleotide identity with the previously characterized IS*407* element of *B. cepacia* (Wood et al., 1991). This homology prompted the designation IS*407*$_{Pa}$, with the subscript $_{Pa}$ to indicate it is the *P. aeruginosa* version. Both elements are similar in size (1243 bp for *IS*407$_{Bc}$ and 1211 for *IS*407$_{Pa}$) and have very similar imperfect inverted repeats (IR) of 12 and 11 bp respectively. The *IS*407 elements are similar to IS sequences from other soil-, water- and plant-associated bacteria, including IS*R1* from *R. meliloti* (Priefer et al., 1989), IS*511* from *Caulobacter crescentens*, IS*1222* from *Enterobacter agglomerans*, IS*476* from *Xanthamonas campestris* (Kearney and Staskawicz, 1990), and IS*911* from *S. dysenteriae* (Prère et al., 1990). There have been previous reports of *IS* elements in *P. aeruginosa* (Pritchard and Vasil, 1990; Sokol et al., 1994) but none of these have homology to the above group; therefore this is the first report of IS*407* in *P. aeruginosa*. Southern blot analysis using the IS*407*$_{Pa}$ as a probe showed

it is present in all 20 serotypes of *P. aeruginosa* (Table 2), and most serotypes appear to have only a single copy f the element.

psbM.

The *psbM* gene follows the $IS407_{Pa}$ element and may be transcribed from one of three potential promoters present in the right IR (Figure 31). A gene-activating promoter was previously shown to be present in the right IR of $IS407_{Bc}$ (Wood et al., 1991). *psbM* is unusual because in contrast to other *psb* genes described above, it hybridiz s to chromosomal DNA from all 20 serotypes (Table 1). PsbM mutants, generated by insertion of a gentamicin cassette into a unique *Nru*I site within *psbM*, exhibit B-band LPS-minus phenotype. This confirms the involvement of the *psbM* product in LPS biosynthesis, despite the fact it lies outside of the O5-specific region (Figure 41). PsbM has homology to a range of proteins involved in exopolysaccharide synthesis, including BplL from the *B. pertussis* LPS cluster (Allen and Maskell, 1996), TrsG from the core biosynthetic cluster of *Y. enterocolitica* O3 (Skurnik et al., 1995), and CapD from the *S. aureus* capsular gene cluster (Lin et al., 1994). These homologies are summarized in Table 2.

As shown previously for BplL, only the carboxy half of the PsbM protein has homology to GalE from several bacterial species, suggesting it may have originated as a fusion protein. In support of this hypothesis, PsbM also has homology to two adjacent ORFs (ORF10 and ORF11) in the LPS cluster of *V. cholerae* O139 (Comstock et al., 1996). The homology to ORF10 and ORF11 lies in the amino-terminal and carboxy-terminal half of PsbM, respectively (Table 2), suggesting that two similar ORFs were fused during the evolution of PsbM and the BplL/TrsG/CapD group.

Based on these homologies, PsbM is thought to be involved in the biosynthesis of the *N*-acetylfucosamine residue of the O5 O-antigen. As mentioned above, the O-antigen of *B. pertussis* and the type 1 capsule of *S. aureus* and the outer core of *Y. enterocolitica* O3 all contain *N*-acetylfucosamine. PsbM could function as a dehydrogenase, and it contains two putative NAD-binding domains (Figure 33), as do BplL and TrsG. Again, these duplications may have arisen from an ancestral fusion of two NAD-binding domain-containing proteins and may be bifunctional.

psbN.

The *psbN* gene has some homology to *eryA*, a gene involved in erythromycin biosynthesis in *Sacchropolyspora erythrae*. Generation of knockout mutations in *psbN* will demonstrate its function in biosynthesis of the O5 O-antigen.

uvrB.

The last partial open reading frame present on pFV100 has high homology to the highly conserved *uvrB* gene from several bacterial species, including *E. coli*, *S. enterica* sv Typhimurium, and Micrococcus luteus. UvrB is a subunit of the UvrABC

DNA excision repair complex involved in removal of thymidine dimers induced by irradiation with ultraviolet light. The presence of *uvrB* adjacent to *psbN* confirms that *psbN* is the last gene in the *psb* cluster that could be involved in O-antigen biosynthesis.

Organization of the *psb* gene cluster in *P. aeruginosa* O5.

Several entire *rfb* clusters, particularly from enteric bacteria, have been characterized to date (reviewed in Whitfield and Valvano, 1993; and Schnaitman and Klena, 1993). In general, *rfb* clusters are located on the chromosome adjacent to the *his* operon and the *gnd* gene. Amongst the enterics, it has previously been shown that the *rfb* clusters are organized in a specific fashion (Reeves, 1993; Schnaitman and Klena, 1993). Genes necessary for sugar biosynthesis are arranged in discrete blocks located 5' to the transferases and other assembly genes (*rfbX*, *rfc* and *rol*). The *psb* cluster, however, appears to be almost randomly organised, with genes thought to be involved in the biosynthesis of Man(2NAc3N)A and Man(2NAc3NAc)A scattered throughout the gene cluster (*psbI*, *psbE*, *psbD*, *psbB* and *psbC*). The genes thought to encode for the biosynthesis of FucNAc are also scattered throughout the cluster (*psbK*, *psbM*, *psbG*, *psbN*). Further, the genes encoding transferases are interspersed throughout the *psb* cluster (*psbH*, *psbJ*, *psbL*), and are separated from one another by one gene each. However, the transferase genes do appear to be organized such that the gene encoding the putative first transferase (PsbL), thought to initiate O-antigen assembly on undecaprenol, is the most distal. Recent results from detailed spectroscopic analysis, using high resolution NMR and Mass Spectroscopy of an *rfc* mutant of PAO1, strain AK1401, show that FucNAc is the first sugar of the O-antigen unit, attached to the core oligosaccharide. PsbL's homology to Rfe, and its hydropathicity support the interpretation that it is the first transferase, and is responsible for attachment of the FucNAc residue to undecaprenol. Therefore, based on their gene order and their relative hydropathic indices (-0.21 and 0.10), the *psbJ* and *psbH* gene products are thought to transfer Man$(NAc)_2$A and Man(2NAc3N)A, respectively.

The O-antigen of *P. aeruginosa* O5 is an Rfc-dependent heteropolymer.

The *psb* cluster was shown to contain an *rfc* gene, (See Example 1) the interruption of which (by knockout mutation and gene replacement) resulted in a SR phenotype (de Kievit et al., 1995). At least two other gene products, Rol and RfbX, are thought to be involved in Rfc-dependent synthesis of heteropolymeric O-antigens (Whitfield, 1994). Here a *rol* gene has been identified in the *psb* cluster. However, in the analysis of the *psb* genes, no *rfbX*-like gene was identified. The *psbF* gene product appeared to be the most likely candidate, based on its hydropathy profile (Figure 9), but insertional mutants of *psbF* do not have the phenotype expected of *rfbX* mutants.

Identification of his genes within the *psb* gene cluster.

The identification of the *hisH* and *hisF* genes in the middle of the *psb* cluster raises some interesting evolutionary questions. It appears that these two *his* genes are not native to *P. aeruginosa*, because they have a lower %G+C content than background (50% vs.67%) and they hybridize only to a limited number of serotypes with related O-antigens instead of all 20 serotypes. It is not uncommon for *his* operons to be located adjacent to *rfb* clusters, and it is likely that the *his* genes were acquired simultaneously with some or all of the *psb* genes. The lack of significant homology with any of the HisF and HisH proteins characterized to date, and particularly with those of other Gram-negative bacteria precludes the use of these genes as evolutionary "luggage tags". The lack of homology with other Gram-negative HisH/F proteins suggests either they came from an as-yet uncharacterized source or that they have been resident in *P. aeruginosa* for a long time. The latter possibility is bolstered by the divergence over time of the O-antigen structures/genes from the ancestral *psb* cluster in the five O5-related serotypes in which these *hisH* and *hisF* genes are found.

The location of *hisH* and *hisF* adjacent to one another is unique in bacteria. The similarity between *hisH* and *hisA* genes, and the usual location of *hisA*, rather than *hisH*, adjacent to *hisF*, raises the possibility that the *P. aeruginosa psb hisH* gene was originally a *hisA* gene that has diverged so as to be more similar to *hisH* than to *hisA*. However, there is precedent for the juxtaposition of *hisH* and *hisF*; in the yeast *Sacchromyces cerevisiae*, the homologues of the *hisH* and *hisF* genes are adjacent, and are fused into one translational unit called HIS7 (Kuenzler et al., 1993). Alternatively, the *hisHF* arrangement may be ancestral to the duplication event which resulted in the *hisHAF* gene order. Another possibility is that the *hisA* gene may have been lost, leaving *hisH* and *hisF* adjacent.

psb gene dissemination amongst the 20 serotypes of *P. aeruginosa*.

The observation that no genes were found in the O5 cluster which hybridize only to chromosomal DNA from serotype O5 and not to the other related serotypes was intriguing. The differences among these five serotypes is confined to changes in the type of linkage between sugars or to the epimer present in the O-antigen, either mannuronic or guluronic acid (Figure 30). These differences could result from variation in transferase activity or in epimerization activity, respectively. Further analysis of the putative transferase activities will be necessary to determine whether there are differences in activity among serotypes despite the obvious homology at the genetic level. It will be interesting to determine whether the introduction of multicopy plasmids containing the O5 transferase genes into the related serotypes will result in an alteration in O-antigen structure that could be detectable with serotype-specific monoclonal antibodies. There is precedence for this, as a *P. aeruginosa* strain PAO1 (serotype O5) phage induced

mutant, strain AK1380, was isolated which was identified as serotype O16 (see Lam et al., 1992, Fig.30; and Kuzio and Kropinski, 1993).

The genetic differences among the five serotypes with related O-antigens are obviously quite minor. Comparison of the DNA sequences of the O2 *rfc* and the O5 *rfc* genes revealed they are very homologous at the nucleotide level).

EXAMPLE 4

Further Characterization of Rol (Wzz) Gene and Region Upstream

In this example the *rol* gene is generally referred to as the *wzz* gene.

The materials and methods used in Example 4 are as follows:

Bacterial strains and plasmids.

The bacterial strains and plasmids used in this study are listed in Table 8. *P. aeruginosa* strains were cultured either on Luria broth or plates or on *Pseudomonas* Isolation Agar (PIA: Difco, Detroit, MI). *E. coli* strains were cultured on Luria broth or plates. Media were supplemented with antibiotics ampicillin, carbenicillin, tetracycline, or gentamicin (all from Sigma, St. Louis, MO) as required, using the concentrations outlined in de Kievit et al., 1995.

DNA methods.

Chromosomal DNA was isolated from *P. aeruginosa* using the method of Goldberg and Ohman, 1984. Plasmid and cosmid DNA was isolated using the Qiagen midi-prep kit (Qiagen Inc., Chatsworth, CA) as directed by the manufacturer. Restriction and modification enzymes were supplied by Gibco/BRL (Gaithersburg, MD), Boehringer Mannheim (Laval, PQ), and/or New England Biolabs (Beverly, MA) and were used as directed by the manufacturers.

Plasmids were introduced into *E. coli* by $CaCl_2$ transformation (Huff et al., 1990) and into *P. aeruginosa* by electroporation using a BioRad (Richmond, CA) Gene Pulser apparatus following manufacturers protocols. *P. aeruginosa* electrocompetent cells were prepared by washing early log phase cells twice for 5 min each in sterile 15% room-temperature glycerol followed by immediate resuspension in the same solution. Cells were either used immediately or frozen at -80°C for future use. Alternatively, plasmids were mobilized into *P. aeruginosa* through biparental mating with *E. coli* SM10 carrying plasmids of interest (Simon et al., 1983).

Construction of plasmids.

The cosmid pFV100, containing the *P. aeruginosa wbp* cluster, was used as a source of DNA for the construction of pFV161 (Fig. 43). An overlapping cosmid, pFV400, was the source of a 2.3-kb *Hin*dIII fragment cloned into pBluescript II SK (pFV401). For DNA sequencing, a 0.8 kb *Hin*dIII-*Xho*I fragment from pFV401 was subcloned into pBluescript II SK (pFV402). A 3.0 kb *Sst*I fragment containing the 5 portion of *wzz* and

upstream sequenc s was cloned from pFV400 into pBluescript II SK (pFV403). For complementation experiments, the 2.3 kb insert of pFV401 was cloned into the *Pseudomonas-E. coli* shuttle vector pUCP26 (Table 14), downstream of the vectors *lacZ* promoter (pFV401-26).

DNA sequencing and analysis.

Using the above plasmids, the DNA sequences of both strands of the pFV401 insert were determined by the GenAlyTiC facility (University of Guelph, Guelph, ON) employing the Taq DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Mississauga, ON) and an Ericomp Model TCX15 Thermal cycler. Oligonucleotide primers were synthesized on an Applied Biosystems model 391 DNA synthesizer and purified as directed by the manufacturer.

DNA sequences were collated and analyzed using GENE RUNNER for Windows (Hastings Software, Newark, NJ), DNAsis for Windows (Hitachi Software, Helixx, Scarborough, ON), and PC/GENE (IntelliGenetics Inc., Mountain View, CA). DNA and protein database searches were performed using the NCBI BLAST network server (Altschul et al., 1990; Gish and States, 1993).

Expression of the Wzz protein.

An *E. coli* S30 extract in vitro protein expression kit (Promega, Madison, WI) was used to examine the product encoded by the O5 *wzz* gene. Column-purified (Qiagen) plasmid DNA of pBluescript II SK, pFV401a (containing the O5 *wzz* gene cloned downstream of the *lacZ* promoter of pBluescript II SK) and pFV401b (containing the same DNA cloned in the opposite orientation) were used as templates in the coupled transcription/translation reaction in the presence of ^{35}S-labelled methionine (Trans35-Label, ICN, Costa Mesa, CA). The labelled proteins were precipitated with acetone, separated on standard discontinuous 12.5% SDS-PAGE along with unstained BioRad low-molecular-weight markers and visualized by autoradiography using ^{35}S-sensitive film (BioMax, Kodak, Toronto, ON).

Preparation and visualization of LPS.

LPS from *P. aeruginosa* was prepared by the method of Hitchcock and Brown, 1983. The LPS preparations were separated on standard discontinuous 12.5% SDS-PAGE gels and visualized by silver staining using the method of Dubray and Bezard, 1982. Alternatively, LPS separated on SDS-PAGE gels was transferred to nitrocellulose and visualized by immunoblotting (Burnete, 1981). Nitrocellulose blots were blocked with 3% skim milk followed by overnight incubation with hybridoma culture supernatants containing MAb MF15-4 (specific for O5 B-band LPS), MAb 18-19 (cross-reactive for O2, O5, and O16 B-band LPS core-plus-one O-antigen unit; 28) or MAb N1F10 (specific for A-band LPS; 30). The second antibody was a goat anti-mouse F(ab)$_2$-alkaline phosphatase

conjugate (Jackson Laboratories, Bio/Can Scientific, Mississauga, ON). The blots were developed using a substrate containing 0.3 mg/ml NBT (Nitro Blue Tetrazolium) and 0.15 mg/ml BCIP (5-bromo-4-chloro-3-indolyl phosphate toluidine) (Sigma) in 0.1 M bicarbonate buffer (pH 9.8).

Creation of *wzz* knockout mutants through gene replacement.

The gene replacement strategy of Schweitzer and Hoang, 1985 was used for generation of knockout mutations in *wzz*. The 2.3 kb *Hin*dIII insert of pFV401 was cloned into pEX100T, a pUC19-based vector containing the *sacB* gene as a selectable marker (pFV401T). An 875 bp gentamicin resistance cassette from the plasmid pUCGM was then cloned into the unique *Xho*I site within the insert (pFV401TGm). Constructs containing the interrupted *wzz* gene were mobilized into *P. aeruginosa* O5 by biparental mating with *E. coli* SM10. Since pEX100T does not replicate in *P. aeruginosa*, selection for gentamicin resistance allows detection of chromosomally-integrated copies of the mutated gene. Determination of sucrose and carbenicillin (Cb) sensitivities distinguishes between merodiploids (sucrose^S, Cb^R) and true recombinants (sucrose^R, Cb^S). The presence of the gentamicin cassette in the chromosomal DNA of *P. aeruginosa* O5 and O16 *wzz* mutants was confirmed by Southern blot analysis (not shown).

RESULTS

Cloning and sequencing of the *P. aeruginosa* O5 *wzz* gene.

Nucleotide sequences with homology to *wzz* from *E. coli*, *Salmonella enterica* sv Typhimurium and *Shigella flexneri* (Bastin et al., 1993; Batchelor et al., 1992; Morona et al., 1995) were identified ending approximately 800 bp upstream of the first gene of the *P. aeruginosa* O5 *wbp* gene cluster, *wbpA* (Fig. 43). The amount of DNA with homology to *wzz* was 479 bp, starting at the *Xho*I cloning site of the insert of pFV100 and ending with a stop codon. Based on the average size (1 kb) of previously characterized *wzz* genes (Bastin et al., 1993; Batchelor et al., 1992; Morona et al., 1995), this sequence represented approximately half of the putative *P. aeruginosa wzz* gene.

A 1.5 kb *Xho*I-*Hin*dIII fragment from pFV161 containing the 3 end of the putative *wzz* gene (Fig. 43) was used as a probe to screen a *P. aeruginosa* O5 cosmid library. One cosmid (pFV400) which hybridized with the probe was isolated. A probe-reactive 2.3 kb *Hin*dIII fragment from pFV400 was subcloned into pBluescript II SK to form pFV401 (Fig. 43).

DNA sequence analysis revealed an open reading frame (ORF) of 1046 base pairs (bp), sufficient to encode a protein of 348 amino acids with a molecular mass of 39.3 kilodaltons (kDa), and an isoelectric point of 6.26. Comparison of the deduced amino acid sequence of the *P. aeruginosa* O5 protein with those in GenBank revealed from 11.5 to 20.0% amino acid identity with Wzz-like proteins of other species (Table 15). *P.*

aeruginosa Wzz also has similarity with proteins thought to be involved in polymerization or export of exopolysaccharide capsules in *E. coli* O8/O9 (13, 15; accession #U39306), *Vibrio cholerae* O139 (4; OtnB, X90547), *Klebsiella pneumoniae* (ORF6, 747665), and *Rhizobium meliloti* (ExoP, Z22636). *P. aeruginosa* Wzz also has similarity with FepE from *E. coli*, thought to be a component of the ferric enterobactin permease (Ozenburger et al., 1987; X74129).

While there is poor primary sequence homology between the Wzz protein of *P. aeruginosa* O5 and related proteins, their predicted secondary structures are similar (Fig. 44). There are conserved hydrophobic regions at both the amino and carboxy termini, and hydrophilic regions in the central portion of the protein. The predicted transmembrane helices in *P. aeruginosa* O5 Wzz are between amino acids 29-49 and 319-339. These hydrophobic regions contain the amino acid residues which are most highly conserved among Wzz-like proteins.

Analysis of the region upstream of *wzz*.

The *wzz* gene is upstream of the *wbp* cluster of *P. aeruginosa* O5. As described in Example 3, most of the genes in this cluster, including *wzz*, are serogroup-specific, and are found only in serotypes O2, O5, O16, O18, and O20. These serotypes have chemically- and structurally-related O antigens (Knirel and Koch et Kov., 1994). Based on Southern blot hybridization results, the 5 end of the serogroup-specific region was previously localized to a 1.9-kb *Sst*I-*Xho*I fragment located 1.1 kb upstream of the 5 end of pFV100. DNA sequence analysis of this fragment revealed a gene with 85% nucleotide identity with the *E. coli* gene *rpsA*, encoding 30S ribosomal protein S1 (Schnier et al., 1982), and a second gene which has 98% identity with *P. aeruginosa himD*, encoding the β subunit of integration host factor (IHF) (Delic-Atree et al., 1995). The *rpsA* and *himD* genes are transcribed in the same direction as *wzz*. These data locate *rpsA* and *himD* adjacent to the *wbp* cluster at 37 minutes on the chromosomal map of *P. aeruginosa* O5 strain PAO1 (Holloway et al., 1994; Lightfoot and Lam, 1993).

Expression of the putative Wzz protein.

Using an *E. coli* S30 extract expression system, the putative *wzz* gene was shown to encode a protein with an apparent molecular weight of 40 kDa which was not present in samples containing only the vector, pBluescript II SK (Fig. 45). The estimated size of 40 kDa is in good agreement with that predicted from the DNA sequence (39.3 kDa). A reduced amount of the same protein was detected in the sample in which the insert DNA was cloned in the opposite orientation (pFV401b), indicating that there is a native promoter present upstream of the *wzz* gene which functions weakly in *E. coli*. Examination of the DNA sequence upstream of *wzz* revealed at least three potential promoter sequences

with partial homology to the *E. coli* δ^{70} consensus. The -10 regions of these putative promoters are located approximately 60, 140, or 155 bp upstream of the *wzz* initiation cod n.

Analysis of the putativ Wzz protein function using chromosomal kn ckout mutants.

A gentamicin-resistance (Gm^R) cassette was inserted into the putative *wzz* gene of *P. aeruginosa* O5, and the interrupted gene was reintroduced into the O5 chromosome by homologous recombination. Comparison of LPS from the wild-type strain and the Gm^R mutant on silver-stained SDS-PAGE gels and Western immunoblots using B-band-specific MAbs MF15-4 and 18-19 showed that the mutant had an altered LPS banding pattern. When MAb 18-19 was used, the LPS from the *wzz* mutant showed an increase in both shorter and longer B-band LPS O chains and a decrease in B-band O chains whose length corresponded to that preferred in the O5 parent strain (Fig. 46). On the immunoblot using MAb MF15-4, which is specific for high-molecular-weight LPS (Lam et al., 1992), there is also an increase in both shorter and longer B-band O chains. Similar Western immunoblots using the A-band LPS-specific MAb N1F10 showed the modality of A-band was unaffected by the *wzz* mutation (not shown). Although the B-band LPS pattern of the *wzz* mutant is significantly different from the parent strain, it does not show the linear distribution of O-antigen chain lengths seen in enteric *wzz* mutants (Fig. 47A). Reintroduction of the O5 *wzz* gene on pFV401-26 restored the mutant to a phenotype similar to that of the parent but missing both the shortest and longest groups of chain lengths (Fig. 46).

Comparison of the function of *wzz* in two related serotypes of *P. aeruginosa*.

A DNA probe containing the O5 *wzz* gene hybridized with chromosomal DNA only from serotypes O2, O5, O16, O18, and O20 of *P. aeruginosa*, all of which have chemically- and structurally-related O antigens (Example 3). The O antigens of both O5 and O16 are composed of two mannuronic acid and one *N*-acetyl fucosamine residues, but differ in one glycosidic linkage. In O5, the linkage is (1(3)-(-D-Fuc2NAc, while in O16, the linkage is (1(3)-(-D-Fuc2NAc. This change results in a discernible difference in the LPS patterns of O5 and O16 (Fig. 46).

Taking advantage of the similarity between the O-antigen gene clusters of O5 and O16, a *wzz* knockout mutation was introduced into O16, using the O5 *wzz* knockout construct. As an additional benefit, O16 does not express A-band LPS (Lam et al., 1989), thus any changes in B-band LPS patterns on silver-stained gels were more easily visualized. The structural difference between O5 and O16 LPS is detected by MAb MF15-4, which recognizes only O5 and not O16 LPS. To examine LPS from both O5 and O16 simultaneously on Western immunoblots, MAb 18-19, which cross-reacts with all five serotypes in the O5 serogroup (Lam et al., 1992), was used. Comparison of LPS from the wild-type O16 parent and the O16 *wzz* knockout mutant showed the mutant displayed a

loss of modality corresponding to the preferred chain lengths of the parent, and an increase in higher-molecular-weight LPS (Fig. 46). Interestingly, there still appeared to be chain length modulation in the O16 *wzz* mutant that was different from that of the parent, with a decrease in short O chains in comparison to the O5 *wzz* mutant. Bastin and coworkers (1996) showed that the modality of chain length distribution was dependent on the source of the *wzz* gene. However, the pattern of LPS chain length distribution of O16 *wzz* mutants carrying the O5 *wzz* gene on pFV401-26 resembled that of the O16 parent strain, rather than the O5 strain (Fig. 46).

Ability of the *P. aeruginosa* O5 *wzz* gene to function in *E. coli*.

In order to determine whether *wzz* from *P. aeruginosa* O5 could complement an enteric *wzz* mutation, *E. coli* strain CLM4, which is deleted for O-antigen genes including *wzz* (Marolda and Valvano, 1993), was used. CLM4 was transformed with either pSS37 (containing the O-antigen biosynthetic genes from *S. dysenteriae* type I without a *wzz* gene alone, or with both pSS37 and pFV401, containing *P. aeruginosa* O5 *wzz*. While LPS from *E. coli* CLM4/pSS37 showed an unregulated distribution of chain lengths, LPS from *E. coli* CLM4/pSS37/pFV401 showed a restoration to modality, with a decrease in short and very long O chains, and an increase in chains with approximately 10-20 repeats (Fig. 47A).

The core oligosaccharide of the *E. coli* K-12 hybrid strain HB101, but not K-12 itself, can act as an acceptor for *P. aeruginosa* O antigens (Goldberg et al., 1992; Lightfoot and Lam, 1993). The structure of the HB101 core has not been elucidated. Although *E. coli* HB101 carrying pFV100 had previously been shown to express LPS which could be recognized by B-band-specific MAb MF15-4, its chain-length regulation had not been examined. pFV100 is now known to contain a truncated *wzz* gene. The expression of LPS from *E. coli* HB101 carrying both pFV100 and the complete O5 *wzz* gene on pFV401 was examined. *E. coli* HB101 carrying pFV100 alone expressed an O5 O antigen with modulated, short-chain O-antigen molecules (Fig. 47B). When both pFV100 and pFV401 were present in *E. coli* HB101, a dual LPS banding pattern was visible on Western immunoblots (Fig. 47B). The coexpression of both *E. coli* and *P. aeruginosa* Wzz proteins resulted in a major group of short O chains attributable to HB101 Wzz, and a minor group with longer chains attributable to the *P. aeruginosa* O5 Wzz protein.

The identification of the *rpsA* and *himD* genes upstream of *wzz* completes the delineation of the region of serogroup-specific DNA responsible for encoding the B-band LPS O antigen of *P. aeruginosa* O5 and related serotypes. The entire O5 *wbp* cluster is thus bounded by *himD* on the 5 end and *uvrB* on the 3 end and is approximately 24.3 kb from the start of *wzz* to the end of *wbpN*. The serogroup-specific portion is approximately 18.4 kb from the start of *wzz* to the end of *wbpL*. Unlike enteric O-antigen

(*rfb*) clusters, the *wbp* cluster is not flanked by *his* and *gnd*, although there are two *his* genes, *hisH* and *hisF*, located in the center of the cluster. The location of *wzz* upstream of the *wbp* cluster in *P. aeruginosa* is opposite to that in many enteric bacteria, where *wzz* is located downstream of the O-antigen cluster (Batchelor et al., 1992; Morona et al., 1995). The presence of the *rpsA* and *himD* genes, which are highly conserved among bacterial species, at the junction between the serogroup-specific and common regions suggests they may have been the site of a past recombination event. *himD* encodes the β-subunit of IHF which has previously been shown to be involved in regulation of biosynthesis of the exopolysaccharide alginate (Wozniak and Ohman, 1993; Wozniak, 1994).

The presence of a functional *wzz* gene in *P. aeruginosa* O5 confirms that both the O-antigen polymerase, Wzy, and Wzz are required for expression of the heteropolymeric B-band O antigen, as predicted by current models. Growing evidence suggests that Wzz proteins may also play a role in the modulation of the length of capsular exopolysaccharide polymers (Bik et al., 1996; Dodgson et al., 1996; Franco et al., 1996). A possible homologue of the third component of Wzy-dependent systems, Wzx, is present in the *wbp* cluster (Burrows et al., 1996).

The LPS banding pattern of enteric *wzz* mutants consists mainly of short O chains with steadily decreasing amounts of longer chains (Fig. 47A). In contrast, neither the O5 nor the O16 *wzz* mutants display this typical *wzz* phenotype, and the O16 mutant in particular continues to display some chain length regulation. It is possible that chain length regulation in *P. aeruginosa* is not simply dependent on *wzz*. In the case of O16, there may be a second *wzz* gene present in the O16 chromosome whose activity is normally masked by the *wzz* of the O5 serogroup. Complementation of the O5 and O16 mutants by *wzz* on a multicopy plasmid gave rise to strains whose LPS appeared even more tightly regulated for size than that of the parent strains, since the complemented *wzz* mutants lacked both short- and very long-chain modal groups, and had an increase in medium-length groups. One possible interpretation of these results is that the regulation of chain length by *wzz* in *P. aeruginosa* is normally imprecise, giving rise to groups with multiples of the preferred chain length instead of a single group. This interpretation fits the model of Bastin et al., 1993 who suggested that multimodal distributions of chain lengths could result from reinitiation of polymerization without an intervening ligation step.

Complementation of the O16 mutants by the O5 *wzz* gene restored them to a phenotype resembling the O16 parent. Contrary to the findings of Bastin and colleagues, 1993, these results show that in these closely-related serotypes, the structure of the O antigen, or possibly difference in the O5 vs O16 genetic background, determines the preferred O-antigen chain length. While the O16 *wzz* and *wzy* genes have not been

isolated, they are probably highly similar to those of O5 based on the results of high-stringency Southern blot analysis. The analysis of *wzy* from the related serotypes O2 and O5 demonstrated that the genes are essentially identical.

The *P. aeruginosa* O5 Wzz protein can modulate expression of both homologous (*P. aeruginosa* O5) and heterologous (*S. dysenteriae*) O antigens in *E. coli* although it has only 20% identity with the Wzz protein of *E. coli*. The ablility of *P. aeruginosa* Wzz to modulate a heterologous O antigen is consistent with previous work showing Wzz is not specific for O-antigen type. When *E. coli* and *P. aeruginosa* Wzz proteins are coexpressed in *E. coli*, the modulating effect of the native protein predominates although the *P. aeruginosa wzz* is present in multicopy. This difference can be seen in the increased proportion of short O chains versus longer O chains which are expressed. Despite variations in efficacy, it appears that the Wzz proteins from different Gram-negative families function in an analogous manner and can act as interchangeable components of the O-antigen assembly complex.

The ability of Wzz, Wzy and WaaL proteins with divergent primary sequences to act reciprocally suggests that they are interacting through recognition of common, conserved structural features. Although the amino acid similarities between the Wzz proteins are low, their secondary structures are alike (Fig. 44). Similarly, although the primary sequence similarities of the Wzy proteins from a number of bacteria are poor, all have highly similar secondary structures containing multiple membrane-spanning domains (Cryz et al., 1984). Comparison of the WaaL proteins from *E. coli* and *S. enterica* sv Typhimurium, the only O-antigen ligases characterized to date, show that they too have conserved secondary structures, but less than 20% primary sequence homology (Liu and Wang, 1990). In light of this information, it is now possible to target conserved structural features of these proteins for modification in order to further define the areas critical for putative protein interactions.

Having illustrated and described the principles of the invention in a preferred embodiment, it should be appreciated to those skilled in the art that the invention can be modified in arrangement and detail without departure from such principles. We claim all modifications coming within the scope of the following claims.

All publications, patents and patent applications referred to herein are incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

Below full citations are set out for the references referred to in the specification and detailed legends for the figures are provided.

The application contains sequence listings which form part of the application.

TABLE 1

***Pseudomonas aeruginosa* serotype O5 *wbp* gene cluster.**

locus	base positions	%G+C	MW encoded	AAs[d]	pI[e]	H.I.[f]	distribution[g]
wzz[a]	1-479	49.5	38.6 kDa	158	nd	nd	2, 5, 16, 18, 20
wbpA	1286-2596	54.5	48.2 kDa	436	5.36	-0.08	2, 5, 16, 18, 20
wbpB	2670-3620	52.8	35.8 kDa	316	6.40	-0.27	2, 5, 16, 18, 20
wbpC	3689-5578	53.1	69.9 kDa	629	9.06	0.48	2, 5, 16, 18, 20
wbpD	5575-6066	53.9	17.4 kDa	163	8.25	0.19	2, 5, 16, 18, 20
wbpE	6152-6982	52.8	29.9 kDa	276	5.26	-0.01	2, 5, 16, 18, 20
wzy[b]	7236-8552	44.6	48.9 kDa	438	9.63	0.80	2, 5, 16, 18, 20
wbpF	8549-9499	49.0	33.8 kDa	316	9.49	0.99	2, 5, 16, 18, 20
hisH	9831-10388	49.3	20.9 kDa	185	nd	nd	2, 5, 16, 18, 20
hisF	10388-11143	50.0	27.5 kDa	251	nd	nd	2, 5, 16, 18, 20
wbpG	11281-12411	44.5	43.4 kDa	376	8.15	-0.38	2, 5, 16, 18, 20
wbpH	12427-13548	45.6	42.0 kDa	373	8.79	-0.21	2, 5, 16, 18, 20
wbpI	13545-14633	50.2	39.7 kDa	362	5.40	0.06	2, 5, 16, 18, 20
wbpJ	14651-15892	54.5	45.3 kDa	413	6.54	0.10	2, 5, 16, 18, 20
wbpK	15889-16851	56.8	34.4 kDa	320	9.03	0.14	2, 5, 16, 18, 20
wbpL[c]	16911-17822	55.5	32.9 kDa	303	9.08	0.84	2, 5, 16, 18, 20
IS*1209*	17935-19144	59.3	nd	n/a	n/a	n/a	1 to 11, 13 to 20
wbpM	19678-21675	61.9	74.5 kDa	665	9.33	0.09	1 to 20
wbpN	22302-23693	63.6	48.5 kDa	463	6.12	-0.09	1 to 20
uvrB[a]	23704-24417	61.2	26.7 kDa	238	nd	nd	1 to 20

[a] truncated ORF

[b] de Kievit et al. (1995)

[c] *wbpL* was originally named *rfbA*; Dasgupta and Lam (1995)

[d] number of amino acids

[e] isoelectric point of the protein, calculated using GeneRunner for Windows (Hastings Software).

[f] hydropathic index of the protein, calculated using DNAsis for Windows (Hitachi Software). Positive values indicate the protein is hydrophobic, while negative values indicate the protein is hydrophilic.

[g] distribution of this gene among the 20 serotypes of *P. aeruginosa*, based on positive hybridization in high-stringency Southern blot analysis.

TABLE 2

Similarities of *P. aeruginosa* O5 Wbp proteins to those in the databases.

P. aeruginosa protein	Similar proteins	Putative function	% identity (% similarity)*	Database accession number
WbpA	EpsD-*Burkholderia solanacearum*	dehydrogenase	33.1 (50.6)	U17898
	CapL-*Staphylococcus aureus*	capsule synthesis	31.6 (45.3)	U10927
	VipA-*Salmonella enterica* sv Typhi	Vi antigen synthesis	30.8 (44.9)	D14156
	RffD (o379)-*Escherichia coli*	UDP-ManNAc dehydrogenase	30.2 (42.8)	M87049
WbpB	LmbZ-*Streptomyces lincolnesis*	oxidoreductase	19.3 (28.2)	X79146
	BplA-*Bordetella pertussis*	dehydrogenase	12.4 (17.0)	X90711
	Pur10-*Str. alboniger*	oxidoreductase	5.7 (12.0)	X92429
WbpC	HI0392-*Haemophilus influenzae*	unknown	24.9 (37.2)	U00073
	ExoZ-*Rhizobium meliloti*	O-acylase	27.4 (40.3)	U50300
	AcyA-*Str. thermotolerans*	O-acylase	24.9 (37.2)	X58126
	unknown-*Caenorhabditis elegans*	unknown	18.0 (26.7)	D30759
	NodX-*R. leguminosarum*	O-acylase	16.3 (23.1)	X07990
WbpD	BplB-*B. pertussis*	acetylase	73.6 (83.4)	X90711
	CysE-*Buchnera aphidicola*	serine O-acetylase	28.2 (45.4)	M90644
	CysE-*Arabidopsis thalnia*	serine O-acetylase	30.7 (42.4)	L42212
	CysE-*H. influenzae*	serine O-acetylase	28.2 (39.9)	U32689
	CysE-*E. coli*	serine O-acetylase	28.8 (38.6)	M15745

TABLE 2 Cont'd

WbpE	BplC-*B. pertussis*	aminase	64.1 (75.7)	X90711
	DegT-*Bacillus subtilis*	dideoxy sugar biosynthesis	51.2 (62.4)	M29002
	ERYC1-*Saccharopolyspora erythrae*	dideoxy sugar biosynthesis	37.3 (48.2)	P14290
	SpsC-*Ba. subtilis*	dideoxy sugar biosynthesis	37.4 (53.3)	P39623
	Dnrj-*Str. peucetius*	dideoxy sugar biosynthesis	34.1 (50.4)	P25048
WbpF	ExoT-*R. meliloti*	succinoglycan export	20.3 (32.3)	Z22646
	FeuC-*Ba. subtilis*	iron uptake	17.1 (28.8)	L19954
WbpG	ORF2-*Vibrio cholerae* O139	unknown	19.0 (23.7)	U47057
	Pfk-*Lactococcus lactis*	phosphofructokinase	9.7 (14.4)	L07920
	NrfB-*H. influenzae*	formyl-dependent nitrate reductase	5.8 (9.3)	U32733
WbpH	RfaK-*Neisseria meningitidis*	glycosyl transferase	20.1 (28.9)	U35713
	CapM-*S. aureus*	GalNAcA transferase	17.4 (29.7)	U10927
	IcsA-*N. meningitidis*	glycosyl transferase	17.1 (27.0)	U39810
	BplH-*B. pertussis*	glycosyl transferase	16.6 (23.0)	X90711
	BplE-*B. pertussis*	glycosyl transferase	15.8 (24.6)	X90711
WbpI	BplD-*B. pertussis*	GlcNAc to ManNAc epimerase	56.6 (69.3)	X90711
	EpsC-*B. solanacearum*	GalNAcA biosynthesis	29.3 (42.3)	U17898
	RffE (o389)-*E. coli*	UDP-GlcNAc-2-epimerase	12.9 (18.8)	M87049
	YvyH-*Ba. subtilis*	unknown	12.3 (18.5)	P39131
	RfbC-*S. enterica* sv Borreze	UDP-GlcNAc-2-epimerase	11.8 (18.2)	L39794
WbpJ	BplE-*B. pertussis*	glycosyl transferase	39.5 (52.2)	X90711
	TrsE-*Yersinia enterocolitica* O:3	galactosyl transferase	15.7 (26.7)	Z47767

TABLE 2 Cont'd

WbpK	ORF6-*V. cholerae* O139	UDP-galactose-4-epimerase	37.2 (53.8)	U47057
	ExoB-*R. meliloti*	UDP-galactose-4-epimerase	22.8 (32.8)	X58126
	StrP-*Str. glaucescens*	dehydratase or epimerase	22.5 (34.7)	X78974
	RffG (o355)-*E. coli*	TDP-glucose dehydratase	25.5 (38.1)	M87049
	GraE-*Str. violaceoruben*	unknown	21.3 (29.7)	L37334
	RfbB-*N. meningitidis*	TDP-glucose dehydratase	21.9 (31.6)	L09189
	RfbB-*E. coli*	TDP-glucose dehydratase	18.8 (28.5)	U23775
WbpL	TrsF-*Y. enterocolitica* O3	UDP-GalNAc transferase	54.5 (67.7)	Z47767
	Rfe-*Mycobacterium leprae*	UDP-GlcNAc transferase	28.7 (46.5)	U15186
	Rfe-*M. tuberculosis*	UDP-GlcNAc transferase	28.5 (46.6)	Z73419
	Rfe-*E. coli*	UDP-GlcNAc transferase	19.8 (30.3)	M76129
	Rfe-*H. influenzae*	UDP-GlcNAc transferase	19.1 (29.7)	U32791
WbpM	BplL-*B. pertussis*	dehydratase	48.4 (59.6)	X90711
	TrsG-*Y. enterocolitica* O3	UDP-GalNAc biosynthesis	48.1 (60.0)	Z47767
	CapD-*S. aureus*	unknown	39.2 (53.9)	U10927
	ORF10-*V. cholerae* O139	unknown	32.5 (52.4)[a]	U47057
	ORF11-*V. cholerae* O139	unknown	52.7 (61.0)[b]	U47057
WbpN	NifV-*Rhodobacter sphaeroides*	homocitrate synthase	19.2 (27.1)	Q01181

TABLE 3

Amino acid homologies of HisH proteins

	PA	AB	EC	HI	LL	SC	ST
PA	100.0	-	-	-	-	-	-
AB	53.6	100.0	-	-	-	-	-
EC	56.1	47.4	100.0	-	-	-	-
HI	51.8	47.9	63.3	100.0	-	-	-
LL	51.0	52.6	50.0	52.3	100.0	-	-
SC	54.9	47.9	55.1	45.2	48.0	100.0	-
ST	54.7	43.2	92.2	60.9	45.4	49.5	100.0

Amino acid homologies of HisH proteins from various bacterial species. The amino acid sequences of various HisH proteins were aligned pairwise using the PC/GENE PALIGN program with the following parameters: K-tuple value = 1; gap penalty = 5; window size = 10; open gap cost = 10; unit gap cost = 10; filtering level = 2.5. The numbers shown are a summation of identical and conserved amino acid residues. Key: PA, *Pseudomonas aeruginosa* O5 *psb* cluster HisH; AB, *Azospirillum brazilense* HisH; EC, *Escherichia coli* HisH; HI, *Haemophilus influenzae* HisH; LL, *Lactobacillus lactis* HisH; RS, *Rhodobacter sphaeroides* HisH; and ST, *Salmonella enterica* typhimurium HisH.

TABLE 4

Amino acid homologies of HisF proteins.

	Pa	Ab	Ec	Hi	Kp	Ll	Rs	St
Pa	100.0	-	-	-	-	-	-	-
Ab	51.4	100.0	-	-	-	-	-	-
Ec	48.2	56.2	100.0	-	-	-	-	-
Hi	50.6	52.3	87.2	100.0	-	-	-	-
Kp	49.8	55.5	97.7	86.4	100.0	-	-	-
Ll	53.7	70.1	58.6	57.0	58.6	100.0	-	-
Rs	44.6	81.3	54.8	46.8	54.0	63.2	100.0	-
St	49.4	56.5	97.3	87.6	96.5	58.6	55.2	100.0

Amino acid homologies of HisF proteins from various bacterial species. The amino acid sequences of various HisF proteins were aligned pairwise using the PC/GENE PALIGN program with the following parameters: K-tuple value = 1; gap penalty = 5; window size = 10; open gap cost = 10; unit gap cost = 10; filtering level = 2.5. The numbers shown are a summation of identical and conserved amino acid residues. Key: Pa, *Pseudomonas aeruginosa* O5 *psb* cluster HisF; Ab, *Azospirillum brazilense* HisF; Ec, *Escherichia coli* HisF; Hi, *Haemophilus influenzae* HisF; Ll, *Lactobacillus lactis* HisF; Rs, *Rhodobacter sphaero4ides* HisF; and St, *Salmonella enterica* typhimurium HisF.

TABLE 5

Pairwise comparison of Rol amino acid homologies[1,2]

	PA	EC1	EC2	SF	ST
PA	100.0	34.4	35.1	35.4	32.8
EC1		100.0	79.3	79.0	78.6
EC2			100.0	98.1	81.5
SF				100.0	81.2
ST					100.0

[1] Analyses were done using PCGENE PALIGN program.

[2] PA, *Pseudomonas aeruginosa* O5 Rol; EC1, *E. coli* O75 Rol; EC2, *E. coli* O111 CLD; SF, *Shigella flexneri* Rol; ST, *Salmonella enterica* serovar typhimurium strain LT2 CLD. N te that CLD (chain length determinant) is another nomenclature used by some researchers (Bastin *et al.*, 1993) to describe the same class of Rol proteins.

TABLE 6

Bacterial strains and plasmids

Strain or plasmid	Genotype or relevant characteristics	Reference or source
P. aeruginosa		
PAO1	serotype O5, A^+, B^+	Hancock and Carey (1979)
AK14O1	mutant of OT684[a], A^+, B-band contains core + one O-repeat unit (SR)	Berry and Kropinski (1986)
rd7513	mutant of AK14O1, A^-, B-band contains core + one O-repeat unit (SR)	Lightfoot and Lam (1991)
OP5.2	mutant of PAO1, A^+, B-band contains core + one O-repeat unit (SR)	This study
OP5.3	mutant of PAO1, A^+, B-band contains core + one O-repeat unit (SR)	This study
OP5.5	mutant of PAO1, A^+, B-band contains core + one O-repeat unit (SR)	This study
E. coli		
DH5	*supE44 hsdR17 recA1 endA1 gyrA96 thi-1 relA1*	GIBCO/Bethesda Research Laboratories
HB101	*supE44 hsdS20*($r^-_B m^-_B$) *recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1* F^- Str^R	Boyer and Roulland-Dussoix (1969)
SM10	*thi-1 thr leu tonA lacY supE recA* RP4-2-Tc::Mu Km^R	Simon *et al.* (1983)
Plasmids		
pFV100	pCP13 derivative containing cloned PAO1 O-antigen biosynthetic genes on a 26 kb insert	Lightfoot and Lam (1993)
pCP13	RK2 derivative *cos*$^+$, Mob^+, Tra^-, Tc^R Km^R	Darzins and Chakrabarty (1984)
pRK404	RK2 derivative Mob^+, Tra^-, Tc^R	Ditta *et al.* (1985)
pUCP26	pUC18-derived broad-host-range vector, Tc^R	West *et al.* (1994)
pEX100T	gene-replacement vector, *oriT*$^+$, *SacB*$^+$, Ap^R	Schweizer and Hoang (submitted)
pUCPGM	source of Gm^R cassette; Ap^R Gm^R	Schweizer (1993)
pBluescript KS (+/-)	Ap^R	PDI Biosciences, Aurora, ON

[a]OT684 is the immediate progenitor strain of AK14O1 and is a restrictionless mutant of PAO1 (Potter and Loutit, 1982).

TABLE 7

Rfc proteins of *P. aeruginosa* and other gram-negative organisms

Rfc protein	Total # amino acids	Mol. weight (kD)[a]	Hydropathy index[b]	% G + C[c]	Reference
P. aeruginosa	438	48.9	0.8	44.8	This study
S. enterica (typhimurium)	407	47.5	0.65	33.5	Collins and Hackett (1991)
S. enterica (muenchen)	399	44.8	0.77	33.8	Brown *et al.* (1992)
Shigella dysenteriae	380	43.7	0.84	30.9	Klena and Schnaitman (1993)
Shigella flexneri	382	43.7	1.08	27.3	Morona *et al.* (1994)

[a]Molecular weight based on nucleotide sequence.

[b]Hydropathy index deduced from hydrophobicity analysis (Kyte and Doolittle, 1982).

[c]Percentage of the bases G and C in the coding sequence.

TABLE 8

Bacterial strains and plasmids used in this study.

Strain or plasmid	Genotype, phenotype or properties	Reference/source
P. aeruginosa		
O5	strain PAO1, wild type A+ B+	20
O5 *wzz*	PAO1, *wzz* insertion mutation at *Xho*I; A+ B+	this study
IATS O16	Serotype O16 wild type A- B+	33
O16 *wzz*	Serotype O16 *wzz* insertion mutation at *Xho*I; A- B+	this study
E. coli		
JM109	*recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi (lac-proAB* F'[*tra* D36, *proAB*$^+$, *lacI*^{q}, *lacZ*.(M15]	53
SM10	*thi-1 thr leu tonA lacY supE recA* RP4-2-Tc::Mu, Km^R	45
HB101	F- *thi-1 hsdS20 serA ara14 proA2 lacY1 galK2 rpsL20 xyl mtl-1 supE44 recA13 leuB6* Str^R	27
CLM4	*lacZ2286 trp-49 ((sbcB-rfb)86 upp-12 relA1 rps1150* (- *recA*	35
Plasmids		
pFV100	24.4 kb *Xho*I fragment in cosmid pCP13; contains the *wbp* cluster	8, 31
pFV400	25.0 kb Sau3A1 fragment in pCP13; overlaps pFV100	this study
pFV401	2.3 kb *Hin*dIII fragment in pBluescript II SK; contains the *P. aeruginosa* O5 *wzz* gene	this study
pFV401-26	same insert in pUCP26	this study
pFV401TGm	same insert in pEX100T, with Gm^R cassette inserted at unique *Xho*I site within *wzz*	this study
pFV403	3.0 kb *Sst*I fragment in pBluescript II SK; contains 5 portion of *wzz* and upstream sequences	this study
pBluescript II SK	2.9 kb cloning vector containing T7 promoter; Ap^R	Stratagene
pUCP26	4.9 kb pUC18-based broad-host-range vector; Tc^R	48
pEX100T	gene-replacement vector; *oriT*$^+$, *sacB*$^+$, Ap^R	44
pUCPGM	source of gentamicin resistance cassette; Ap^R, Gm^R	44

TABLE 9

Amino acid identities/similarities of various *wzz*-like proteins.

	Ec Wzz	Ec o349	Sf Wzz	St Wzz	Ec O8 Wzz	Ye Wzz	Yp Wzz	Ec FepE	Vc OtnB
Pa Wzz	19.9 (33.4)	15.5 (26.5)	20.0 (35.4)	19.6 (32.8)	19.3 (32.9)	11.5 (19.0)	13.2 (23.3)	17.0 (27.3)	18.8 (30.4)
Ec Wzz	100.0	25.1 (35.8)	65.5 (79.0)	64.8 (78.6)	65.2 (80.4)	19.3 (27.3)	22.6 (35.4)	26.9 (39.4)	18.7 (28.4)
Ec o349	-	100.0	20.3 (32.0)	24.8 (37.6)	21.2 (33.9)	14.7 (22.7)	20.7 (31.9)	19.5 (31.3)	18.5 (26.3)
Sf Wzz	-	-	100.0	72.0 (81.2)	88.9 (93.6)	15.7 (25.9)	20.9 (33.5)	24.6 (36.6)	18.8 (25.0)
St Wzz	-	-	-	100.0	71.2 (82.6)	15.6 (23.6)	22.6 (33.3)	26.6 (41.9)	22.6 (32.7)
Ec O8 Wzz	-	-	-	-	100.0	15.2 (26.0)	15.5 (26.9)	24.7 (36.1)	15.2 (26.3)
Ye Wzz	-	-	-	-	-	100.0	37.3 (56.9)	25.1 (38.4)	10.4 (19.7)
Yp Wzz	-	-	-	-	-	-	100.0	36.1 (51.8)	18.2 (29.2)
Ec FepE	-	-	-	-	-	-	-	100.0	14.0 (24.2)

Numbers shown are percent identity, with percent similarity in brackets.
Pa, *P. aeruginosa* O5, accession U50397; Ec Wzz, *E. coli* O111, Z17241; Ec o349, *E. coli*, M87049; Sf Wzz, *Shigella flexneri*, X71970; St Wzz, *S. enterica* sv Typhimurium LT2, M89933; Ec O8 Wzz, *E. coli* O8, U39306; Ye Wzz, *Yersinia enterocolitica* O:8, U43708; Yp Wzz, *Y. pseudotuberculosis*, U13685; Ec FepE, *E. coli*, P26266; Vc OtnB, *Vibrio cholerae* O139, X90547.

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Detailed Figure Legends for Figures 22 to 29, 32, 33, and 43 to 47

Figure 22. Silver-stained SDS-PAGE gel of LPS from PAO1, AK14O1, AK14O1(pFV100), and AK14O1(pFV.TK8) (Panel A) and Westernimmunoblots of this LPS reacted with O5-specific MAb MF15-4 (Panel B) . Note that the two transconjugants strains, AK14O1(pFV100) and AK14O1(pFV.TK8), produce levels of B-band LPS similar to the PAO1 wild-type strain.

Figure 23. Restriction maps of the chromosomal inserts from pFV100 and several pFV subclones. Results of complementation studies of the SR mutants AK14O1 and rd7513 with the pFV subclones are also shown. The three Tn*1000* insertions in the 1.5 kb *Xho*I fragment of pFV.TK6 that were found to interrupt O-antigen complementation in AK14O1 are indicated. This *Xho*I fragment was later purified and used as a probe in Southern blot analysis. Restriction sites: B, *Bam*HI; X, *Xho*I; S, *Spe*I; Xb, *Xba*I; H, *Hin*dIII.

Figure 24. Southern analysis the three *rfc* chromosomal mutants, OP5.2, OP5.3, and OP5.5, showing the insertion of an 875 bp Gm^R cassette into the *rfc* gene. Restriction maps of the PAO1 wild-type (panel A) and mutant (panel B) *rfc* coding regions are shown. Southern hybridizations of chromosomal DNA from PAO1 (lane 1) and mutants OP5.2, OP5.3, and OP5.5 (lanes 2-4, respectively) digested with *Xho*I were performed using an *rfc* probe (panel C). This DIG-labelled probe was generated from the 1.5 kb *Xho*I insert of pFV.TK7 (shown in panel A). The probe hybridized to a 1.5 kb fragment of PAO1 and a 2.4 kb fragment of thethree *rfc* mutants. The molecular size of the probe-reactive fragments are shown on the left (in kb) .

Figure 25. Silver-stained SDS-PAGE gel and Western blots of LPS from PAO1, AK14O1 and the three *rfc* chromosomal mutants, OP5.2, OP5.3, and OP5.5. Panel A: silver-stained SDS-PAGE gel; Panel B: Western blot reacted with O5-specific MAb MF15-4; Panel C: Western blot reacted with A-band specific MAb N1F10. Note that the chromosomal *rfc* mutants are not able to produce long-chain O-antigen; however, they are still expressing A-band LPS, like the SR mutant AK14O1.

Figure 26. Restriction maps of recombinant plasmids pFV161, pFV401 and pFV402. The shaded box represents the DIG-labeled probe generated from pFV161. Restriction sites: B, *Bam*HI; H, *Hin*dIII; X, *Xho*I.

Figure 27. Southern hybridizations of chromosomal DNA from PAO1 (lane 2) and *rol* mutants (lanes 3&4). Chromosomal DNA in Panel A was digested with *Pst*I and *Sst*I. DNA

in Panel B was digested with *Hin*dIII. The samples in Panel A were probed with the Gm^R cassette (Schweizer, 1993). The probe used in Panel B is the 2.3 kb *Hin*dIII insert from pFV401. Molecular weight markers, using λ DNA digested with *Hin*dIII, are indicated to the left of each panel.

Figure 28. Characterization of LPS from PAO1 and PAO1 *rol* chromosomal mutants. The samples in each lane are as labeled. Panel A is a silver-stained SDS-PAGE gel. Panel B is the corresponding Western immunoblot reacted with an O5 (B-band)-specific mAb MF15-4.

Figure 29. T7 protein expression of *P. aeruginosa* O5 Rol. This autoradiogram shows ^{35}S-labeled proteins expressed by pFV401, which contains the *rol* gene, and corresponding control plasmid vector pBluescript II SK in *E. coli* JM109DE3 by use of the T7 expression system. The arrow indicates the putative Rol protein. Molecular size markers are indicated to the left of the figure.

Figure 32. Features of the initiation regions. Capital letters for bases indicate one of the following sites: potential ribosomal binding sites (RBS), the presumed start codon (also in bold and double underlined), the second codon where it is AAA (the preferred second codon), and components of the sequences TTAA and AAA from +10 to +13 and from -1 to -3 respectively (Gold and Stormo, 1987). The termination codon of the preceding gene is indicated by a bar above if it is in the region shown. The reference sequences involved are also shown above the set of sequences.

Figure 33. NAD-binding domains of PsbA, PsbK and PsbM aligned with those of other bacterial proteins involved in polysaccharide biosynthesis. The consensus sequence for an NAD-binding domain (Macpherson et al., 1994) is shown at the bottom of the figure in bold underline. The first column contains the protein names; the second column indicates the location of the NAD-binding site within the protein; the third column shows the alignment of the NAD-binding domains with highly conserved residues indicated in bold type; and the fourth column gives the reference for the protein shown. Most of the proteins in this group of sugar biosynthesis enzymes function as dehydrogenases/dehydratases. Note that PsbM, BplL, and TrsG have two putative NAD-binding domains, instead of one. The presence of two domains supports the proposal that these large proteins arose from fusion of two smaller proteins.

Figure 43. Physical map of the 5 end of the *wbp* cluster. The *wzz* gene ends approximately 800 bp upstream of *wbpA*, the first gene of the *wbp* cluster (8). The probe used to identify a *Hin*dIII fragment containing the intact *wzz* gene for cloning into pFV401 is shown as a black

bar above the restriction map. The site of insertion of the gentamicin cassette used to create the *wzz* knockout mutants is indicated by a black triangle. Key: B, *Bam*HI; H, *Hin*dIII; S, *Sst*I; X, *Xho*I.

Figure 44. Comparison of hydropathy plots of selected Wzz-like proteins. The hydropathy plots of selected Wzz-like proteins were calculated using PC/GENE SOAP. The X axis represents amino acid residues, while the Y axis represents relative hydropathy. Positive values indicate hydrophobicity; negative values indicate hydrophilicity. A, *P. aeruginosa* O5 Wzz, U50397; B, *E. coli* O111 Wzz, Z17241; C, *E. coli* o349, M87049; D, *E. coli* FepE, P26266; E, *Y. enterocolitica* O8 Wzz, U43708; F, *Y. pseudotuberculosis* Wzz, ; G, *V. cholerae* O139 OtnB, X90547.

Figure 45. Expression of *P. aeruginosa* Wzz in vitro. The 40 kDa Wzz protein (indicated by black arrowhead) was expressed from the insert of pFV401 in both orientations. A 28 kDa protein was also expressed in both orientations and may represent either a breakdown product of the 40 kDa polypeptide, or initiation of translation from a secondary ribosome-binding site. There are several smaller ORFs encoded on the positive strand of the 2.3 kb insert of pFV401 which could correspond to the 10 kDa protein.

Figure 46. Analysis of LPS from *wzz* knockout mutants. LPS from *P. aeruginosa* serotypes O5 and O16 and their corresponding *wzz* mutants was examined. Figure 46A: Silver-stained 12.5% SDS-PAGE. Figure 46B: Western immunoblot using MAb 18-19, specific for B-band LPS from the O5 serogroup (serotypes O2, O5, O16, O18, O20). Figure 46C: Western immunoblot using MAb MF15-4, specific for serotype O5 B-band LPS. The plasmid pFV401-26 contains the O5 *wzz* gene cloned downstream of the *lacZ* promoter of shuttle vector pUCP26.

Figure 47. Ability of *P. aeruginosa* O5 Wzz to function in *E. coli*.
Panel A. Silver-stained SDS-PAGE gel of *E. coli* CLM4 containing the *Shigella dysenteriae rfb* cluster on pSS37, with and without the *P. aeruginosa wzz* gene in pFV401. Panel B. Western immunoblot of *E. coli* HB101 containing the *P. aeruginosa* O5 *wbp* cluster in pFV100, with and without the *P. aeruginosa wzz* gene in pFV401. The membrane was incubated with MAb MF15-4, specific for serotype O5 B-band LPS.

Figure 48. Western immunoblot analysis of lipopolysaccharide (LPS) isolated using the hot water-phenol method of Westphal and Jann. Lanes O5 are LPS from the parent strain, while lanes F1 and F2 are LPS from two mutants containing a gentamicin cassette inserted at

the *Sst*I site within the open reading frame of *wbpF*. The monoclonal antibodies used are N1F10, specific for A-band LPS, and 18-19, specific for B-band LPS. Note that a knockout mutation of *wbpF* abrogates both A-band and B-band LPS expression.

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(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24417 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Pseudomonas aeruginosa
(B) STRAIN: PA01

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GCGGCGTCTT CCTCGATGCC GTTGCCGAAG CCCTGGTAGG CGATGTCGAG GAACGGCACG 22980

TGGCCCTTGG CCTTGAGCAC GTCCAGCACC TGTTTCCAGT CGTCCAGCTC GAGATCGACG 23040

CCGGTCGGAT TATGGCAGCA GGCGTGCAGA ACCACGATCG AGCGGGCCGG CAGGGCATTC 23100

AGGTCTTCCA GCAGGCCGGC GCGGTTCACG CCATTGCTGG CGGCGTCGTA ATAGCGGTAG 23160

TTCTGCACCG GGAAGCCGGC GGCTTCGAAC AGTGCGCGGT GGTTTTCCCA GCTCGGGTCG 23220

CTGATGGCCA CGGTGGCGTC GGGCAGCAGG CGCTTGAGGA AGTCGGCGCC GAGCTTGAGC 23280

GCGCCGGTGC CGCCGACGGC CTGGGTCGTG ACCACACGGC CGGCGGCCAG CAGCTCGGAC 23340

TCGTTACCGA ACAGCAGTTT CTGTACGCCC TGGTCGTAGG CGGCGATCCC TTCGATCGGC 23400

AGGTAGCCGC GCGGCGCGTG GGCCTCGATG CGGGCCTTCT CGGCAGCCTG CACGGCACGC 23460

AACAGCGGAA TGCGCCCCTC CTCGTTGTAG TACACGCCCA CGCCCAGGTT GATCTTGCCC 23520

GGACGGGTAT CGGCGTTGAA GGCTTCGTTC AGGCCAAGGA TGGGATCACG CGGTGCCATT 23580

TCGACGGCAG AAAACAGACT CATTTTGCGG CTGCTCGGAG TGTGAAGAGA GGAGGGCAAC 23640

GCAACCCGTT ATGCGGGGGC GCAAAGGGTT GCGCAAACGG GGGGTTATTA TAGACACCCC 23700

TTGATGCATG CGGCGACATT TAGGTGCATG CTTTCAGCTA TTTCTGACGC CGGATTTTCC 23760

TTGGCGTCAC AGCTCCCTGC GAGGTTTTTC ATGGATACGT TCCAACTCGA CTCGCGCTTC 23820

AAGCCCGCCG GCGACCAGCC GGAAGCCATC CGGCAAATGG TCGAGGGGCT GGAGGCGGGG 23880

CTTTCGCACC AGACCCTGCT GGGGGTGACG GGCTCTGGCA AGACTTTCAG CATCGCCAAC 23940

GTGATTGCCC AGGTGCAGCG CCCGACCCTG GTCCTGGCGC CGAACAAGAC CCTGGCGGCC 24000

CAGCTCTACG GGGAGTTCAA GACGTTCTTC CCGCACAATT CCGTGGAGTA CTTCGTTTCC 24060

TACTACGACT ACTACCAGCC GGAGGCCTAC GTCCCGTCTT CCGATACCTA TATCGAGAAG 24120

GACTCCTCGA TCAACGACCA TATCGAGCAG ATGCGCCTGT CGGCGACCAA GGCGCTGCTC 24180

GAGCGTCCGG ATGCGATCAT CGTCGCCACC GTGTCGTCCA TCTACGGCCT CGGTGATCCC 24240

GCGTCCTACC TGAAGATGGT CCTGCACCTG GACCGCGGCG ACCGCATCGA CCAGCGCGAA 24300

CTGCTGCGGC GACTGACCAG CCTGCAGTAC ACCCGCAACG ACATGGATTT CGCCCGTGCG 24360

ACTTTCCGTG TGCGTGGCGA TGTGATCGAC ATCTTCCCGG CCGAATCCGA TCTCGAG 24417

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 158 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Pseudomonas aeruginosa
(B) STRAIN: PA01

(vii) IMMEDIATE SOURCE:
(B) CLONE: rol

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Arg Asp Ile Glu Gln Arg Ile Gln Asn Leu Arg Arg Glu Cys Gln Gly
1 5 10 15

Arg Arg Glu Asp Arg Ile Val Gln Leu Lys Glu Ala Leu Lys Val Ala
20 25 30

Gly Ala Leu Lys Leu Glu Glu Pro Pro Leu Ile Ser Gly Gln Ser Ser
35 40 45

Glu Glu Leu Ser Ala Ile Met Asn Gly Ser Leu Met Tyr Met Arg Gly
50 55 60

Ser Lys Ala Ile Met Ala Glu Ile Gln Thr Leu Glu Ala Arg Ser Ser
65 70 75 80

Asp Asp Pro Phe Ile Pro Ala Leu Arg Thr Leu Gln Glu Gln Gln Leu
85 90 95

Leu Leu Ser Ser Leu Arg Val Asn Ser Glu Arg Val Ser Val Phe Arg
100 105 110

Gln Asp Gly Pro Ile Glu Thr Pro Asp Ser Pro Val Arg Pro Arg Arg
115 120 125

Ala Met Ile Leu Ile Phe Gly Leu Ile Ile Gly Gly Val Leu Gly Gly
130 135 140

Phe Leu Ala Leu Cys Arg Ile Phe Leu Lys Lys Tyr Ala Arg
145 150 155

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 436 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Pseudomonas aeruginosa
(B) STRAIN: PA01

(vii) IMMEDIATE SOURCE:
(B) CLONE: psbA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Ile Asp Val Asn Thr Val Val Glu Lys Phe Lys Ser Arg Gln Ala
1 5 10 15

Leu Ile Gly Ile Val Gly Leu Gly Tyr Val Gly Leu Pro Leu Met Leu
20 25 30

Arg Tyr Asn Ala Ile Gly Phe Asp Val Leu Gly Ile Asp Ile Asp Asp
35 40 45

Val Lys Val Asp Lys Leu Asn Ala Gly Gln Cys Tyr Ile Glu His Ile
50 55 60

Pro Gln Ala Lys Ile Ala Lys Ala Arg Ala Ser Gly Phe Glu Ala Thr
65 70 75 80

Thr Asp Phe Ser Arg Val Ser Glu Cys Asp Ala Leu Ile Leu Cys Val
85 90 95

Pro Thr Pro Leu Asn Lys Tyr Arg Glu Pro Asp Met Ser Phe Val Ile
100 105 110

Asn Thr Thr Asp Ala Leu Lys Pro Tyr Leu Arg Val Gly Gln Val Val
115 120 125

Ser Leu Glu Ser Thr Thr Tyr Pro Gly Thr Thr Glu Glu Glu Leu Leu
130 135 140

Pro Arg Val Gln Glu Gly Gly Leu Val Val Gly Arg Asp Ile Tyr Leu
145 150 155 160

Val Tyr Ser Pro Glu Arg Glu Asp Pro Gly Asn Pro Asn Phe Glu Thr
165 170 175

Arg Thr Ile Pro Lys Val Ile Gly Gly His Thr Pro Gln Cys Leu Glu
180 185 190

Val Gly Ile Ala Leu Tyr Glu Gln Ala Ile Asp Arg Val Val Pro Val
195 200 205

Ser Ser Thr Lys Ala Ala Glu Met Thr Lys Leu Leu Glu Asn Ile His
210 215 220

Arg Ala Val Asn Ile Gly Leu Val Asn Glu Met Lys Ile Val Ala Asp
225 230 235 240

Arg Met Gly Ile Asp Ile Phe Glu Val Val Asp Ala Ala Ala Thr Lys
245 250 255

Pro Phe Gly Phe Thr Pro Tyr Tyr Pro Gly Pro Gly Leu Gly Gly His
260 265 270

Cys Ile Pro Ile Asp Pro Phe Tyr Leu Thr Trp Lys Ala Arg Glu Tyr
275 280 285

Gly Leu His Thr Arg Phe Ile Glu Leu Ser Gly Glu Val Asn Gln Ala
290 295 300

Met Pro Glu Tyr Val Leu Gly Lys Leu Met Asp Gly Leu Asn Glu Ala
305 310 315 320

Gly Arg Ala Leu Lys Gly Ser Arg Val Leu Val Leu Gly Ile Ala Tyr
325 330 335

Lys Lys Asn Val Asp Asp Met Arg Glu Ser Pro Ser Val Glu Ile Met
340 345 350

Glu Leu Ile Glu Ala Lys Gly Gly Met Val Ala Tyr Ser Asp Pro His
355 360 365

Val Pro Val Phe Pro Lys Met Arg Glu His His Phe Glu Leu Ser Ser
370 375 380

Glu Pro Leu Thr Ala Glu Asn Leu Ala Arg Phe Asp Ala Val Val Leu
385 390 395 400

Ala Thr Asp His Asp Lys Phe Asp Tyr Glu Leu Ile Lys Ala Glu Ala
405 410 415

Lys Leu Val Val Asp Ser Arg Gly Lys Tyr Arg Ser Pro Ala Ala His
420 425 430

Ile Ile Lys Ala
435

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 316 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Pseudomonas aeruginosa
(B) STRAIN: PA01

(vii) IMMEDIATE SOURCE:
(B) CLONE: psbB

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Lys Asn Phe Ala Leu Ile Gly Ala Ala Gly Tyr Ile Ala Pro Arg
1 5 10 15

His Met Arg Ala Ile Lys Asp Thr Gly Asn Cys Leu Val Ser Ala Tyr
20 25 30

Asp Ile Asn Asp Ser Val Gly Ile Ile Asp Ser Ile Ser Pro Gln Ser
35 40 45

Glu Phe Phe Thr Glu Phe Glu Phe Phe Leu Asp His Ala Ser Asn Leu
50 55 60

Lys Arg Asp Ser Ala Thr Ala Leu Asp Tyr Val Ser Ile Cys Ser Pro
65 70 75 80

Asn Tyr Leu His Tyr Pro His Ile Ala Ala Gly Leu Arg Leu Gly Cys
85 90 95

Asp Val Ile Cys Glu Lys Pro Leu Val Pro Thr Pro Glu Met Leu Asp
100 105 110

Gln Leu Ala Val Ile Glu Arg Glu Thr Asp Lys Arg Leu Tyr Asn Ile
115 120 125

Leu Gln Leu Arg His His Gln Ala Ile Ile Ala Leu Lys Asp Lys Val
130 135 140

Ala Arg Glu Lys Ser Pro His Lys Tyr Glu Val Asp Leu Thr Tyr Ile
145 150 155 160

Thr Ser Arg Gly Asn Trp Tyr Leu Lys Ser Trp Lys Gly Asp Pro Arg
165 170 175

Lys Ser Phe Gly Val Ala Thr Asn Ile Gly Val His Phe Tyr Asp Met
180 185 190

Leu His Phe Ile Phe Gly Lys Leu Gln Arg Asn Val Val His Phe Thr
195 200 205

Ser Glu Tyr Lys Thr Ala Gly Tyr Leu Glu Tyr Glu Gln Ala Arg Val
210 215 220

Arg Trp Phe Leu Ser Val Asp Ala Asn Asp Leu Pro Glu Ser Val Lys

225 230 235 240

Gly Lys Lys Pro Thr Tyr Arg Ser Ile Thr Val Asn Gly Glu Glu Met
245 250 255

Glu Phe Ser Glu Gly Phe Thr Asp Leu His Thr Thr Ser Tyr Glu Glu
260 265 270

Ile Leu Ala Gly Arg Gly Tyr Gly Ile Asp Asp Ala Arg His Cys Val
275 280 285

Glu Thr Val Asn Thr Ile Arg Ser Ala Val Ile Val Pro Ala Ser Asp
290 295 300

Asn Glu Gly His Pro Phe Val Ala Ala Leu Ala Arg
305 310 315

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 766 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Pseudomonas aeruginosa
(B) STRAIN: PA01

(vii) IMMEDIATE SOURCE:
(B) CLONE: psbC

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Leu Cys Thr Ser Leu Pro Ser Thr Arg Gln Leu Val Ile Trp Ser
1 5 10 15

Thr Ser Arg Pro Val Cys Val Gly Phe Cys Pro Trp Met Leu Thr Thr
20 25 30

Cys Arg Ser Arg Ser Arg Ala Lys Ser Arg Pro Ile Val Arg Leu Pro
35 40 45

Ser Thr Val Arg Lys Trp Ser Ser Leu Lys Ala Leu Pro Ile Tyr Ile
50 55 60

Gln Pro Ala Thr Lys Lys Phe Ser Leu Val Val Met Ala Ser Met
65 70 75 80

Thr Leu Val Ile Val Trp Lys Leu Ser Ile Pro Phe Ala Ala Pro Ser
85 90 95

Ser Tyr Arg Pro Leu Ile Thr Lys Gly Ile Arg Ser Ser Arg Arg Leu
100 105 110

Arg Val Glu Val Glu Lys Glu Trp Pro Ser Ser Val Thr Cys Leu Gln
115 120 125

Gln Val Ser Ala Gly Ser Phe Ile Ser Met Ser Ser Ser Ser Ser Lys
130 135 140

Leu Leu Asn Gly Met Val Ala Val Ser Ser Gly Arg Asn Ile Arg Leu
145 150 155 160

Asp Val Gln Gly Leu Arg Ala Val Ala Val Leu Ala Val Leu Ala Tyr
165 170 175

His Ala Asn Ser Ala Trp Leu Arg Ala Gly Phe Val Gly Val Asp Val
180 185 190

Phe Phe Val Ile Ser Gly Phe Ile Ile Thr Ala Leu Leu Val Glu Arg
195 200 205

Gly Val Lys Val Asp Leu Val Glu Phe Tyr Ala Gly Arg Ile Lys Arg
210 215 220

Ile Phe Pro Ala Tyr Phe Val Met Leu Ala Ile Val Cys Ile Val Ser
225 230 235 240

Thr Ile Leu Phe Leu Pro Asp Asp Tyr Val Phe Phe Glu Lys Ser Leu
245 250 255

Gln Ser Ser Val Phe Phe Ser Ser Asn His Tyr Phe Ala Asn Phe Gly
260 265 270

Ser Tyr Phe Ala Pro Arg Ala Glu Glu Leu Pro Leu Leu His Thr Cys
275 280 285

Ser Ile Ala Asn Glu Met Gln Phe Tyr Leu Phe Tyr Pro Val Leu Phe
290 295 300

Met Cys Leu Pro Cys Arg Trp Arg Leu Pro Val Phe Ile Leu Leu Ala
305 310 315 320

Ile Leu Leu Phe Ile Trp Ser Gly Tyr Cys Val Phe Ser Gly Ser Gln
325 330 335

Asp Ala Gln Tyr Phe Ala Leu Leu Ala Arg Val Pro Glu Phe Met Ser
340 345 350

Gly Ala Val Val Ala Leu Ser Leu Arg Asp Arg Glu Leu Pro Ala Arg
355 360 365

Leu Ala Ile Leu Ala Gly Leu Leu Gly Ala Ala Leu Leu Val Cys Ser
370 375 380

Phe Ile Ile Ile Asp Lys Gln His Phe Pro Gly Phe Trp Ser Leu Leu
385 390 395 400

Pro Cys Leu Gly Ala Ala Leu Leu Ile Ala Ala Arg Arg Gly Pro Ala
405 410 415

Ser Leu Leu Leu Ala Ser Arg Pro Met Val Trp Ile Gly Gly Ile Ser
420 425 430

Tyr Ser Leu Tyr Leu Trp His Trp Pro Ile Leu Ala Phe Ile Arg Tyr
435 440 445

Tyr Thr Gly Gln Tyr Glu Leu Ser Phe Val Ala Leu Leu Ala Phe Leu
450 455 460

Thr Gly Ser Phe Leu Leu Ala Trp Phe Ser Tyr Arg Tyr Ile Glu Thr
465 470 475 480

Pro Ala Arg Lys Ala Val Gly Leu Arg Gln Gln Ala Leu Lys Trp Met
485 490 495

Leu Ala Ala Ser Val Val Ala Ile Val Val Thr Gly Gly Ala Gln Phe
500 505 510

Asn Val Leu Val Val Ala Pro Ala Pro Ile Gln Leu Thr Arg Tyr Ala
515 520 525

Val Pro Glu Ser Ile Cys His Gly Val Gln Val Gly Glu Cys Lys Arg
530 535 540

Gly Ser Val Asn Ala Val Pro Arg Val Leu Val Ile Gly Asp Ser His
545 550 555 560

Ala Ala Gln Leu Asn Tyr Phe Phe Asp Val Val Gly Asn Glu Ser Gly
565 570 575

Val Ala Tyr Arg Val Leu Thr Gly Ser Ser Cys Val Pro Ile Pro Ala
580 585 590

Phe Asp Leu Glu Arg Leu Pro Arg Trp Ala Arg Lys Pro Cys Gln Ala
595 600 605

Gln Ile Asp Ala Val Ala Gln Ser Met Leu Asn Phe Asp Lys Ile Ile
610 615 620

Val Ala Gly Met Trp Gln Tyr Gln Met Gln Ser Pro Ala Phe Ala Gln
625 630 635 640

Ala Met Arg Ala Phe Leu Val Asp Thr Ser Tyr Ala Gly Lys Gln Val
645 650 655

Ala Leu Leu Gly Gln Ile Pro Met Phe Glu Ser Asn Val Gln Arg Val
660 665 670

Arg Arg Phe Arg Glu Leu Gly Leu Ser Ala Pro Leu Val Ser Ser Ser
675 680 685

Trp Gln Gly Ala Asn Gln Leu Leu Arg Ala Leu Ala Glu Gly Ile Pro
690 695 700

Asn Val Arg Phe Met Asp Phe Ser Ser Ser Ala Phe Phe Ala Asp Ala
705 710 715 720

Pro Tyr Gln Asp Gly Glu Leu Ile Tyr Gln Asp Ser His His Leu Asn
725 730 735

Glu Val Gly Ala Arg Arg Tyr Gly Tyr Phe Ala Ser Arg Gln Leu Gln
740 745 750

Arg Leu Phe Glu Gln Pro Gln Ser Ser Val Ser Leu Lys Pro
755 760 765

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 160 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Pseudomonas aeruginosa
(B) STRAIN: PA01

(vii) IMMEDIATE SOURCE:
(B) CLONE: psbD

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ser Tyr Tyr Gln His Pro Ser Ala Ile Val Asp Asp Gly Ala Gln
1 5 10 15

Ile Gly Ser Asp Ser Arg Val Trp His Phe Val His Ile Cys Ala Gly
20 25 30

Ala Arg Ile Gly Ala Gly Val Ser Leu Gly Gln Asn Val Phe Val Gly
35 40 45

Asn Lys Val Val Ile Gly Asp Arg Cys Lys Ile Gln Asn Asn Val Ser
50 55 60

Val Tyr Asp Asn Val Thr Leu Glu Glu Gly Val Phe Cys Gly Pro Ser
65 70 75 80

Met Val Phe Thr Asn Val Tyr Asn Pro Arg Ser Leu Ile Glu Arg Lys
85 90 95

Asp Gln Tyr Arg Asn Thr Leu Val Lys Lys Gly Ala Thr Leu Gly Ala
100 105 110

Asn Cys Thr Ile Val Cys Gly Val Thr Ile Gly Glu Tyr Ala Phe Leu
115 120 125

Gly Ala Gly Ala Val Ile Asn Lys Asn Val Pro Ser Tyr Ala Leu Met
130 135 140

Val Gly Val Pro Ala Arg Gln Ile Gly Trp Ile Ala Asn Ser Val Ser
145 150 155 160

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 276 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Pseudomonas aeruginosa
(B) STRAIN: PA01

(vii) IMMEDIATE SOURCE:
(B) CLONE: psbE

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Ile Glu Phe Ile Asp Leu Lys Asn Gln Gln Ala Arg Ile Lys Asp
1 5 10 15

Lys Ile Asp Ala Gly Ile Gln Arg Val Leu Arg His Gly Gln Tyr Ile
20 25 30

Leu Gly Pro Glu Val Thr Glu Leu Glu Asp Arg Leu Ala Asp Phe Val
35 40 45

Gly Ala Lys Tyr Cys Ile Ser Cys Ala Asn Gly Thr Asp Ala Leu Gln
50 55 60

Ile Val Gln Met Ala Leu Gly Val Gly Pro Gly Asp Glu Val Ile Thr

65 70 75 80

Pro Gly Phe Thr Tyr Val Ala Thr Ala Glu Thr Val Ala Leu Leu Gly
85 90 95

Ala Lys Pro Val Tyr Val Asp Ile Asp Pro Arg Thr Tyr Asn Leu Asp
100 105 110

Pro Gln Leu Leu Glu Ala Ala Ile Thr Pro Arg Thr Lys Ala Ile Ile
115 120 125

Pro Val Ser Leu Tyr Gly Gln Cys Ala Asp Phe Asp Ala Ile Asn Ala
130 135 140

Ile Ala Ser Lys Tyr Gly Ile Pro Val Ile Glu Asp Ala Ala Gln Ser
145 150 155 160

Phe Gly Ala Ser Tyr Lys Gly Lys Arg Ser Cys Asn Leu Ser Thr Val
165 170 175

Ala Cys Thr Ser Phe Phe Pro Ser Lys Pro Leu Gly Cys Tyr Gly Asp
180 185 190

Gly Gly Ala Ile Phe Thr Asn Asp Asp Glu Leu Ala Thr Ala Ile Arg
195 200 205

Gln Ile Ala Arg His Gly Gln Asp Arg Arg Tyr His His Ile Arg Val
210 215 220

Gly Val Asn Ser Arg Leu Asp Thr Leu Gln Ala Ala Ile Leu Leu Pro
225 230 235 240

Lys Leu Glu Ile Phe Glu Glu Glu Ile Ala Leu Arg Gln Lys Val Ala
245 250 255

Ala Glu Tyr Asp Leu Ser Leu Lys Gln Val Gly Ile Gly Thr Pro Phe
260 265 270

Ile Gly Ser Gly
275

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 438 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Pseudomonas aeruginosa
(B) STRAIN: PA01

(vii) IMMEDIATE SOURCE:
(B) CLONE: rfc a

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Tyr Ile Leu Ala Arg Val Asp Arg Ser Ile Leu Leu Asn Thr Val
1 5 10 15

Leu Leu Phe Ala Phe Phe Ser Ala Thr Val Trp Val Asn Asn Asn Tyr

20 25 30

Ile Tyr His Leu Tyr Asp Tyr Met Gly Ser Ala Lys Lys Thr Val Asp
35 40 45

Phe Gly Leu Tyr Pro Tyr Leu Met Val Leu Ala Leu Ile Cys Ala Leu
50 55 60

Leu Cys Gly Gly Ala Ile Arg Arg Pro Gly Asp Leu Leu Val Thr Leu
65 70 75 80

Leu Val Val Ile Leu Val Pro His Ser Leu Val Leu Asn Gly Ala Asn
85 90 95

Gln Tyr Ser Pro Asp Ala Gln Pro Trp Ala Gly Val Pro Leu Ala Ile
100 105 110

Ala Phe Gly Ile Leu Ile Ile Gly Ile Val Asn Lys Ile Arg Phe His
115 120 125

Pro Leu Gly Ala Leu Gln Arg Glu Asn Gln Gly Arg Arg Met Leu Val
130 135 140

Leu Leu Ser Val Leu Asn Ile Val Val Leu Val Phe Ile Phe Phe Lys
145 150 155 160

Ser Ala Gly Tyr Phe Ser Phe Asp Phe Ala Gly Gln Tyr Ala Arg Arg
165 170 175

Ala Leu Ala Arg Glu Val Phe Ala Ala Gly Ser Ala Asn Gly Tyr Leu
180 185 190

Ser Ser Ile Gly Thr Gln Ala Phe Phe Pro Val Leu Phe Ala Trp Gly
195 200 205

Val Tyr Arg Arg Gln Trp Phe Tyr Leu Val Leu Gly Ile Val Asn Ala
210 215 220

Leu Val Leu Trp Gly Ala Phe Gly Gln Lys Tyr Pro Phe Val Val Leu
225 230 235 240

Phe Leu Ile Tyr Gly Leu Met Val Tyr Phe Arg Arg Phe Gly Gln Val
245 250 255

Arg Val Ser Trp Val Val Cys Ala Leu Leu Met Leu Leu Leu Leu Gly
260 265 270

Ala Leu Glu His Glu Val Phe Gly Tyr Ser Phe Leu Asn Asp Tyr Phe
275 280 285

Leu Arg Arg Ala Phe Ile Val Pro Ser Thr Leu Leu Gly Ala Val Asp
290 295 300

Gln Phe Val Ser Gln Phe Gly Ser Asn Tyr Tyr Arg Asp Thr Leu Leu
305 310 315 320

Gly Ala Leu Leu Gly Gln Gly Arg Thr Glu Pro Leu Ser Phe Arg Leu
325 330 335

Gly Thr Glu Ile Phe Asn Asn Pro Asp Met Asn Ala Asn Val Asn Phe
340 345 350

Phe Ala Ile Ala Tyr Met Gln Leu Gly Tyr Val Gly Val Met Ala Glu
355 360 365

Ser Met Leu Val Gly Gly Ser Val Val Leu Met Asn Phe Leu Phe Ser

370 375 380

Arg Tyr Gly Ala Phe Met Ala Ile Pro Val Ala Leu Leu Phe Thr Thr
385 390 395 400

Lys Ile Leu Glu Gln Pro Leu Leu Thr Val Met Leu Gly Ser Gly Val
405 410 415

Phe Leu Ile Leu Leu Phe Leu Ala Leu Ile Ser Phe Pro Leu Lys Met
420 425 430

Ser Leu Gly Lys Thr Leu
435

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 316 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Pseudomonas aeruginosa
(B) STRAIN: PA01

(vii) IMMEDIATE SOURCE:
(B) CLONE: psbF

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Ser Ala Ala Phe Ile Asn Arg Val Ala Arg Val Leu Val Gly Thr
1 5 10 15

Leu Gly Ala Gln Leu Ile Thr Ile Gly Val Thr Leu Leu Leu Val Arg
20 25 30

Leu Tyr Ser Pro Ala Glu Met Gly Ala Phe Ser Val Trp Leu Ser Phe
35 40 45

Ala Thr Ile Phe Ala Val Val Val Thr Gly Arg Tyr Glu Leu Ala Ile
50 55 60

Phe Ser Thr Arg Glu Glu Gly Glu Leu Gln Ala Ile Val Lys Leu Ile
65 70 75 80

Leu Gln Leu Thr Leu Leu Ile Phe Val Ala Val Ala Ile Ala Val Val
85 90 95

Ile Gly Arg His Leu Ile Glu Ser Met Pro Val Val Ile Gly Glu Tyr
100 105 110

Trp Phe Ala Leu Ala Val Ala Ser Leu Gly Leu Gly Ile Asn Lys Leu
115 120 125

Val Leu Ser Leu Leu Thr Phe Gln Gln Ser Phe Asn Arg Leu Gly Val
130 135 140

Ala Arg Val Ser Leu Ala Ala Cys Ile Ala Val Ala Gln Val Ser Ala
145 150 155 160

Ala Tyr Leu Leu Glu Gly Val Ser Gly Leu Ile Tyr Gly Gln Leu Phe
165 170 175

Gly Val Val Val Ala Thr Ala Leu Ala Ala Leu Trp Val Gly Lys Ser
180 185 190

Leu Ile Leu Asn Cys Ile Glu Thr Pro Trp Arg Met Val Arg Gln Val
195 200 205

Ala Val Gln Tyr Ile Asn Phe Pro Lys Phe Ser Leu Pro Ala Asp Leu
210 215 220

Val Asn Thr Val Ala Ser Gln Val Pro Val Ile Leu Leu Ala Ala Lys
225 230 235 240

Phe Gly Gly Asp Ser Ala Gly Trp Phe Ala Leu Thr Leu Lys Ile Met
245 250 255

Gly Ala Pro Ile Ser Leu Leu Ala Ala Ser Val Leu Asp Val Phe Lys
260 265 270

Glu Gln Ala Ala Arg Asp Tyr Arg Glu Phe Gly Asn Cys Arg Gly Ile
275 280 285

Phe Leu Lys Thr Phe Arg Leu Leu Ala Val Leu Ala Leu Pro Pro Phe
290 295 300

Ile Ile Phe Gly Ser Leu Ala Ser Gly Pro Leu Gly
305 310 315

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 118 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Pseudomonas aeruginosa
(B) STRAIN: PA01

(vii) IMMEDIATE SOURCE:
(B) CLONE: hisH

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Leu Gly Leu Arg Ser Glu Glu Gly Ala Glu Pro Gly Leu Gly Trp
1 5 10 15

Ile Asp Met Asp Ser Val Arg Phe Glu Arg Arg Asp Asp Arg Lys Val
20 25 30

Pro His Met Gly Trp Asn Gln Val Ser Pro Gln Leu Glu His Pro Ile
35 40 45

Leu Ser Gly Ile Asn Glu Gln Ser Arg Phe Tyr Phe Val His Ser Tyr
50 55 60

Tyr Met Val Pro Lys Asp Pro Asp Asp Ile Leu Leu Ser Cys Asn Tyr
65 70 75 80

Gly Gln Lys Phe Thr Ala Ala Val Ala Arg Asp Asn Val Phe Gly Phe
85 90 95

Gln Phe His Pro Glu Lys Ser His Lys Phe Gly Met Gln Leu Phe Lys
100 105 110

Asn Phe Val Glu Leu Val
115

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 251 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Pseudomonas aeruginosa
(B) STRAIN: PA01

(vii) IMMEDIATE SOURCE:
(B) CLONE: hisF

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Val Arg Arg Arg Val Ile Pro Cys Leu Leu Leu Lys Asp Arg Gly
1 5 10 15

Leu Val Lys Thr Val Lys Phe Lys Glu Pro Lys Tyr Val Gly Asp Pro
20 25 30

Ile Asn Ala Ile Arg Ile Phe Asn Glu Lys Glu Val Asp Glu Leu Ile
35 40 45

Leu Leu Asp Ile Asp Ala Ser Arg Leu Asn Gln Glu Pro Asn Tyr Glu
50 55 60

Leu Ile Ala Glu Val Ala Gly Glu Cys Phe Met Pro Ile Cys Tyr Gly
65 70 75 80

Gly Gly Ile Lys Thr Leu Glu His Ala Glu Lys Ile Phe Ser Leu Gly
85 90 95

Val Glu Lys Val Ser Ile Asn Thr Ala Ala Leu Met Asp Leu Ser Leu
100 105 110

Ile Arg Arg Ile Ala Asp Lys Phe Gly Ser Gln Ser Val Val Gly Ser
115 120 125

Ile Asp Cys Arg Lys Gly Phe Trp Gly Gly His Ser Val Phe Ser Glu
130 135 140

Asn Gly Thr Arg Asp Met Lys Arg Ser Pro Leu Glu Trp Ala Gln Ala
145 150 155 160

Leu Glu Glu Ala Gly Val Gly Glu Ile Phe Leu Asn Ser Ile Asp Arg
165 170 175

Asp Gly Val Gln Lys Gly Phe Asp Asn Ala Leu Val Glu Asn Ile Ala
180 185 190

Ser Asn Val His Val Pro Val Ile Ala Cys Gly Gly Ala Gly Ser Ile
195 200 205

Ala Asp Leu Ile Asp Leu Phe Glu Arg Thr Cys Val Ser Ala Val Ala

210 215 220

Ala Gly Ser Leu Phe Val Phe His Gly Lys His Arg Ala Val Leu Ile
225 230 235 240

Ser Tyr Pro Asp Val Asn Lys Leu Asp Val Gly
245 250

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 376 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Pseudomonas aeruginosa
(B) STRAIN: PA01

(vii) IMMEDIATE SOURCE:
(B) CLONE: psbG

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Lys Ile Cys Ser Arg Cys Val Met Asp Thr Ser Asp Ala Glu Ile
1 5 10 15

Val Phe Asp Glu Ala Gly Val Cys Asn His Cys His Lys Phe Asp Asn
20 25 30

Val Gln Ser Arg Gln Leu Phe Ser Asp Ala Ser Gly Glu Gln Arg Leu
35 40 45

Gln Lys Ile Ile Gly Gln Ile Lys Lys Asp Gly Ser Gly Lys Asp Tyr
50 55 60

Asp Cys Ile Ile Gly Leu Ser Gly Gly Val Asp Ser Ser Tyr Leu Ala
65 70 75 80

Val Lys Val Lys Asp Leu Gly Leu Arg Pro Leu Val Val His Val Asp
85 90 95

Ala Gly Trp Asn Ser Glu Leu Ala Val Ser Asn Ile Glu Lys Ile Val
100 105 110

Lys Tyr Cys Gly Phe Asp Leu His Thr His Val Ile Asn Trp Glu Glu
115 120 125

Ile Arg Asp Leu Gln Leu Ala Tyr Met Lys Ala Ala Val Ala Asn Gln
130 135 140

Asp Val Pro Gln Asp His Ala Phe Phe Ala Ser Met Tyr His Phe Ala
145 150 155 160

Val Lys Asn Asn Ile Lys Tyr Ile Leu Ser Gly Gly Asn Leu Ala Thr
165 170 175

Glu Ala Val Phe Pro Asp Thr Trp His Gly Ser Ala Met Asp Ala Ile
180 185 190

Asn Leu Lys Ala Ile His Lys Lys Tyr Gly Glu Arg Pro Leu Arg Asp
195 200 205

```
Tyr Lys Thr Ile Ser Phe Leu Glu Tyr Tyr Phe Trp Tyr Pro Phe Val
    210                 215                 220

Lys Gly Met Arg Thr Val Arg Pro Leu Asn Phe Met Ala Tyr Asp Lys
225                 230                 235                 240

Ala Lys Ala Glu Thr Phe Leu Gln Glu Thr Ile Gly Tyr Arg Ser Tyr
                245                 250                 255

Ala Arg Lys His Gly Glu Ser Ile Phe Thr Lys Leu Phe Gln Asn Tyr
            260                 265                 270

Tyr Leu Pro Thr Lys Phe Gly Tyr Asp Lys Arg Lys Leu His Tyr Ser
        275                 280                 285

Ser Met Ile Leu Ser Gly Gln Met Thr Arg Asp Glu Ala Gln Ala Lys
    290                 295                 300

Leu Ala Glu Pro Leu Tyr Asp Ala Asp Glu Leu Gln Phe Asp Ile Glu
305                 310                 315                 320

Tyr Phe Cys Lys Lys Met Arg Ile Thr Gln Ala Gln Phe Glu Glu Leu
                325                 330                 335

Met Asn Ala Pro Val His Asp Tyr Ser Glu Phe Ala Asn Trp Asp Ser
            340                 345                 350

Arg Gln Arg Ile Ala Lys Lys Val Gln Met Ile Val Gln Arg Ala Leu
        355                 360                 365

Gly Arg Arg Ile Asn Val Tyr Ser
    370                 375
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 373 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Pseudomonas aeruginosa
(B) STRAIN: PA01

(vii) IMMEDIATE SOURCE:
(B) CLONE: psbH

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Met Thr Lys Val Ala His Leu Thr Ser Val His Ser Arg Tyr Asp Ile
1               5                   10                  15

Arg Ile Phe Arg Lys Gln Cys Arg Thr Leu Ser Gln Tyr Gly Tyr Asp
            20                  25                  30

Val Tyr Leu Val Val Ala Asp Gly Lys Gly Asp Glu Val Lys Asp Gly
        35                  40                  45

Val Arg Ile Val Asp Val Gly Val Leu Ser Gly Arg Leu Asn Arg Ile
    50                  55                  60
```

Leu Lys Thr Thr Arg Lys Ile Tyr Glu Gln Ala Leu Ala Leu Gly Ala
65 70 75 80

Asp Val Tyr His Phe His Asp Pro Glu Leu Ile Pro Val Gly Leu Arg
85 90 95

Leu Lys Lys Gln Gly Lys Gln Val Ile Phe Asp Ser His Glu Asp Val
100 105 110

Pro Lys Gln Leu Leu Ser Lys Pro Tyr Met Arg Pro Phe Leu Arg Arg
115 120 125

Val Val Ala Val Leu Phe Ser Cys Tyr Glu Lys Tyr Ala Cys Pro Lys
130 135 140

Leu Asp Ala Val Leu Thr Ala Thr Pro His Ile Arg Glu Lys Phe Lys
145 150 155 160

Asn Ile Asn Gly Asn Val Leu Asp Ile Asn Asn Phe Pro Met Leu Gly
165 170 175

Glu Leu Asp Ala Met Val Pro Trp Ala Ser Lys Lys Thr Glu Val Cys
180 185 190

Tyr Val Gly Gly Ile Thr Ser Ile Arg Gly Val Arg Glu Val Val Lys
195 200 205

Ser Leu Glu Cys Leu Lys Ser Ser Ala Arg Leu Asn Leu Val Gly Lys
210 215 220

Phe Ser Glu Pro Glu Ile Glu Lys Glu Val Arg Ala Leu Lys Gly Trp
225 230 235 240

Asn Ser Val Asn Glu His Gly Gln Leu Asp Arg Glu Asp Val Arg Arg
245 250 255

Val Leu Gly Asp Ser Val Ala Gly Leu Val Thr Phe Leu Pro Met Pro
260 265 270

Asn His Val Asp Ala Gln Pro Asn Lys Met Phe Glu Tyr Met Ser Ser
275 280 285

Gly Ile Pro Val Ile Ala Ser Asn Phe Pro Leu Trp Arg Glu Ile Val
290 295 300

Glu Gly Ser Asn Cys Gly Ile Cys Val Asp Pro Leu Ser Pro Ala Ala
305 310 315 320

Ile Ala Glu Ala Ile Asp Tyr Leu Val Ser Asn Pro Cys Glu Ala Ala
325 330 335

Ala Leu Gly Arg Asn Gly Gln Arg Ala Val Asn Glu Arg Tyr Asn Trp
340 345 350

Asp Leu Glu Gly Arg Lys Leu Ala Arg Phe Tyr Ser Asp Leu Leu Ser
355 360 365

Lys Arg Asp Ser Ile
370

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 362 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Pseudomonas aeruginosa
(B) STRAIN: PA01

(vii) IMMEDIATE SOURCE:
(B) CLONE: psbI

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Lys Ile Leu Thr Ile Ile Gly Ala Arg Pro Gln Phe Ile Lys Ala
1 5 10 15

Ser Val Val Ser Lys Ala Ile Ile Glu Gln Gln Thr Leu Ser Glu Ile
20 25 30

Ile Val His Thr Gly Gln His Phe Asp Ala Asn Met Ser Glu Ile Phe
35 40 45

Phe Glu Gln Leu Gly Ile Pro Lys Pro Asp Tyr Gln Leu Asp Ile His
50 55 60

Gly Gly Thr His Gly Gln Met Thr Gly Arg Met Leu Met Glu Ile Glu
65 70 75 80

Asp Val Ile Leu Lys Glu Lys Pro His Arg Val Leu Val Tyr Gly Asp
85 90 95

Thr Asn Ser Thr Leu Ala Gly Ala Leu Ala Ala Ser Lys Leu His Val
100 105 110

Pro Ile Ala His Ile Glu Ala Gly Leu Arg Ser Phe Asn Met Arg Met
115 120 125

Pro Glu Glu Ile Asn Arg Ile Leu Thr Asp Gln Val Ser Asp Ile Leu
130 135 140

Phe Cys Pro Thr Arg Val Ala Ile Asp Asn Leu Lys Asn Glu Gly Phe
145 150 155 160

Glu Arg Lys Ala Ala Lys Ile Val Asn Val Gly Asp Val Met Gln Asp
165 170 175

Ser Ala Leu Phe Phe Ala Gln Arg Ala Thr Ser Pro Ile Gly Leu Ala
180 185 190

Ser Gln Asp Gly Phe Ile Leu Ala Thr Leu His Arg Ala Glu Asn Thr
195 200 205

Asp Asp Pro Val Arg Leu Thr Ser Ile Val Glu Ala Leu Asn Glu Ile
210 215 220

Gln Ile Asn Val Ala Pro Val Val Leu Pro Leu His Pro Arg Thr Arg
225 230 235 240

Gly Val Ile Glu Arg Leu Gly Leu Lys Leu Glu Val Gln Val Ile Asp
245 250 255

Pro Val Gly Tyr Leu Glu Met Ile Trp Leu Leu Gln Arg Ser Gly Leu
260 265 270

Val Leu Thr Asp Ser Gly Gly Val Gln Lys Glu Ala Phe Phe Phe Gly

275 280 285

Lys Pro Cys Val Thr Met Arg Asp Gln Thr Glu Trp Val Glu Leu Val
290 295 300

Thr Cys Gly Ala Asn Val Leu Val Gly Ala Ala Arg Asp Met Ile Val
305 310 315 320

Glu Ser Ala Arg Thr Ser Leu Gly Lys Thr Ile Gln Asp Asp Gly Gln
325 330 335

Leu Tyr Gly Gly Gly Gln Ala Ser Leu Gly Leu Leu Asn Ile Leu Pro
340 345 350

Ser Cys Asp Ala Leu Arg Val Glu Phe Lys
355 360

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 413 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Pseudomonas aeruginosa
(B) STRAIN: PA01

(vii) IMMEDIATE SOURCE:
(B) CLONE: psbJ

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Met Asn Val Trp Tyr Val His Pro Tyr Ala Gly Gly Pro Gly Val Gly
1 5 10 15

Arg Tyr Trp Arg Pro Tyr Tyr Phe Ser Lys Phe Trp Asn Gln Ala Gly
20 25 30

His Arg Ser Val Ile Ile Ser Ala Gly Tyr His His Leu Leu Glu Pro
35 40 45

Asp Glu Lys Arg Ser Gly Val Thr Cys Val Asn Gly Ala Glu Tyr Ala
50 55 60

Tyr Val Pro Thr Leu Arg Tyr Leu Gly Asn Gly Val Gly Arg Met Leu
65 70 75 80

Ser Met Leu Ile Phe Thr Met Met Leu Leu Pro Phe Cys Leu Ile Leu
85 90 95

Ala Leu Lys Arg Gly Thr Pro Asp Ala Ile Ile Tyr Ser Ser Pro His
100 105 110

Pro Phe Gly Val Val Ser Cys Trp Leu Ala Ala Arg Leu Leu Gly Ala
115 120 125

Lys Phe Val Phe Glu Val Arg Asp Ile Trp Pro Leu Ser Leu Val Glu
130 135 140

Leu Gly Gly Leu Lys Ala Asp Asn Pro Leu Val Arg Val Thr Gly Trp

```
145                 150                 155                 160

Ile Glu Arg Phe Ser Tyr Ala Arg Ala Asp Lys Ile Ile Ser Leu Leu
                165                 170                 175

Pro Cys Ala Glu Pro His Met Ala Asp Lys Gly Leu Pro Ala Gly Lys
            180                 185                 190

Phe Leu Trp Val Pro Asn Gly Val Asp Ser Ser Asp Ile Ser Pro Asp
        195                 200                 205

Ser Ala Val Ser Ser Ser Asp Leu Val Arg His Val Gln Val Leu Lys
    210                 215                 220

Glu Gln Gly Val Phe Val Val Ile Tyr Ala Gly Ala His Gly Glu Pro
225                 230                 235                 240

Asn Ala Leu Glu Gly Leu Val Arg Ser Ala Gly Leu Leu Arg Glu Arg
                245                 250                 255

Gly Ala Ser Ile Arg Ile Ile Leu Val Gly Lys Gly Glu Cys Lys Glu
            260                 265                 270

Gln Leu Lys Ala Ile Ala Ala Gln Asp Ala Ser Gly Leu Val Glu Phe
        275                 280                 285

Phe Asp Gln Gln Pro Lys Glu Thr Ile Met Ala Val Leu Lys Leu Ala
    290                 295                 300

Ser Ala Gly Tyr Ile Ser Leu Lys Ser Glu Pro Ile Phe Arg Phe Gly
305                 310                 315                 320

Val Ser Pro Asn Lys Leu Trp Asp Tyr Met Leu Val Gly Leu Pro Val
                325                 330                 335

Ile Phe Ala Cys Lys Ala Gly Asn Asp Pro Val Ser Asp Tyr Asp Cys
            340                 345                 350

Gly Val Ser Ala Asp Pro Asp Ala Pro Glu Asp Ile Thr Ala Ala Ile
        355                 360                 365

Phe Arg Leu Leu Leu Leu Ser Glu Asp Glu Arg Arg Thr Met Gly Gln
    370                 375                 380

Arg Gly Arg Asp Ala Val Leu Glu His Tyr Thr Tyr Glu Ser Leu Ala
385                 390                 395                 400

Leu Gln Val Leu Asn Ala Leu Ala Asp Gly Arg Ala Ala
                405                 410
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 320 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Pseudomonas aeruginosa
(B) STRAIN: PA01

(vii) IMMEDIATE SOURCE:
(B) CLONE: psbK

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Lys Ala Val Met Val Thr Gly Ala Ser Gly Phe Val Gly Ser Ala
1 5 10 15

Leu Cys Cys Glu Leu Ala Arg Thr Gly Tyr Ala Val Ile Ala Val Val
20 25 30

Arg Arg Val Val Glu Arg Ile Pro Ser Val Thr Tyr Ile Glu Ala Asp
35 40 45

Leu Thr Asp Pro Ala Thr Phe Ala Gly Glu Phe Pro Thr Val Asp Cys
50 55 60

Ile Ile His Leu Ala Gly Arg Ala His Ile Leu Thr Asp Lys Val Ala
65 70 75 80

Asp Pro Leu Ala Ala Phe Arg Glu Val Asn Arg Asp Ala Thr Val Arg
85 90 95

Leu Ala Thr Arg Ala Leu Glu Ala Gly Val Lys Arg Phe Val Phe Val
100 105 110

Ser Ser Ile Gly Val Asn Gly Asn Ser Thr Arg Gln Gln Ala Phe Asn
115 120 125

Glu Asp Ser Pro Ala Gly Pro His Ala Pro Tyr Ala Ile Ser Lys Tyr
130 135 140

Glu Ala Glu Gln Glu Leu Gly Thr Leu Leu Arg Gly Lys Gly Met Glu
145 150 155 160

Leu Val Val Val Arg Pro Pro Leu Ile Tyr Ala Asn Asp Ala Pro Gly
165 170 175

Asn Phe Gly Arg Leu Leu Lys Leu Val Ala Ser Gly Leu Pro Leu Pro
180 185 190

Leu Asp Gly Val Arg Asn Ala Arg Ser Leu Val Ser Arg Arg Asn Ile
195 200 205

Val Gly Phe Leu Ser Leu Cys Ala Glu His Pro Asp Ala Ala Gly Glu
210 215 220

Leu Phe Leu Val Ala Asp Gly Glu Asp Val Ser Ile Ala Gln Met Ile
225 230 235 240

Glu Ala Leu Ser Arg Gly Met Gly Arg Arg Pro Ala Leu Phe Thr Phe
245 250 255

Pro Ala Val Leu Leu Lys Leu Val Met Cys Leu Leu Gly Lys Ala Ser
260 265 270

Met His Glu Gln Leu Cys Gly Ser Leu Gln Val Asp Ala Ser Lys Ala
275 280 285

Arg Arg Leu Leu Gly Trp Val Pro Val Glu Thr Ile Gly Ala Gly Leu
290 295 300

Gln Ala Ala Gly Arg Glu Tyr Ile Leu Arg Gln Arg Glu Arg Arg Lys
305 310 315 320

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 665 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Pseudomonas aeruginosa
(B) STRAIN: PA01

(vii) IMMEDIATE SOURCE:
(B) CLONE: psbM

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
Met Leu Asp Asn Leu Arg Ile Lys Leu Leu Gly Leu Pro Arg Arg Tyr
1               5                  10                  15

Lys Arg Met Leu Gln Val Ala Ala Asp Val Thr Leu Val Trp Leu Ser
            20                  25                  30

Leu Trp Leu Ala Phe Leu Val Arg Leu Gly Thr Glu Asp Met Ile Ser
        35                  40                  45

Pro Phe Ser Gly His Ala Trp Leu Phe Ile Ala Ala Pro Leu Val Ala
    50                  55                  60

Ile Pro Leu Phe Ile Arg Phe Gly Met Tyr Arg Ala Val Met Arg Tyr
65                  70                  75                  80

Leu Gly Asn Asp Ala Leu Ile Ala Ile Ala Lys Ala Val Thr Ile Ser
                85                  90                  95

Ala Leu Val Leu Ser Leu Leu Val Tyr Trp Tyr Arg Ser Pro Pro Ala
            100                 105                 110

Val Val Pro Arg Ser Leu Val Phe Asn Tyr Trp Trp Leu Ser Met Leu
        115                 120                 125

Leu Ile Gly Gly Leu Arg Leu Ala Met Arg Gln Tyr Phe Met Gly Asp
    130                 135                 140

Trp Tyr Ser Ala Val Gln Ser Val Pro Phe Leu Asn Arg Gln Asp Gly
145                 150                 155                 160

Leu Pro Arg Val Ala Ile Tyr Gly Ala Gly Ala Ala Ala Asn Gln Leu
                165                 170                 175

Val Ala Ala Leu Arg Leu Gly Arg Ala Met Arg Pro Val Ala Phe Ile
            180                 185                 190

Asp Asp Asp Lys Gln Ile Ala Asn Arg Val Ile Ala Gly Leu Arg Val
        195                 200                 205

Tyr Thr Ala Lys His Ile Arg Gln Met Ile Asp Glu Thr Gly Ala Gln
    210                 215                 220

Glu Val Leu Leu Ala Ile Pro Ser Ala Thr Arg Ala Arg Arg Arg Glu
225                 230                 235                 240

Ile Leu Glu Ser Leu Glu Pro Phe Pro Leu His Val Arg Ser Met Pro
                245                 250                 255
```

Gly Phe Met Asp Leu Thr Ser Gly Arg Val Lys Val Asp Asp Leu Gln
260 265 270

Glu Val Asp Ile Ala Asp Leu Leu Gly Arg Asp Ser Val Ala Pro Arg
275 280 285

Lys Glu Leu Leu Glu Arg Cys Ile Arg Gly Gln Val Val Met Val Thr
290 295 300

Gly Ala Gly Gly Ser Ile Gly Ser Glu Leu Cys Arg Gln Ile Met Ser
305 310 315 320

Cys Ser Pro Ser Val Leu Ile Leu Phe Glu His Ser Glu Tyr Asn Leu
325 330 335

Tyr Ser Ile His Gln Glu Leu Glu Arg Arg Ile Lys Arg Glu Ser Leu
340 345 350

Ser Val Asn Leu Leu Pro Ile Leu Gly Ser Val Arg Asn Pro Glu Arg
355 360 365

Leu Val Asp Val Met Arg Thr Trp Lys Val Asn Thr Val Tyr His Ala
370 375 380

Ala Ala Tyr Lys His Val Pro Ile Val Glu His Asn Ile Ala Glu Gly
385 390 395 400

Val Leu Asn Asn Val Ile Gly Thr Leu His Ala Val Gln Ala Ala Val
405 410 415

Gln Val Gly Val Gln Asn Phe Val Leu Ile Ser Thr Asp Lys Ala Val
420 425 430

Arg Pro Thr Asn Val Met Gly Ser Thr Lys Arg Leu Ala Glu Met Val
435 440 445

Leu Gln Ala Leu Ser Asn Glu Ser Ala Pro Leu Leu Phe Gly Asp Arg
450 455 460

Lys Asp Val His His Val Asn Lys Thr Arg Phe Thr Met Val Arg Phe
465 470 475 480

Gly Asn Val Leu Gly Ser Ser Gly Ser Val Ile Pro Leu Phe Arg Glu
485 490 495

Gln Ile Lys Arg Gly Gly Pro Val Thr Val Thr His Pro Ser Ile Thr
500 505 510

Arg Tyr Phe Met Thr Ile Pro Glu Ala Ala Gln Leu Val Ile Gln Ala
515 520 525

Gly Ser Met Gly Gln Gly Gly Asp Val Phe Val Leu Asp Met Gly Pro
530 535 540

Pro Val Lys Ile Leu Glu Leu Ala Glu Lys Met Ile His Leu Ser Gly
545 550 555 560

Leu Ser Val Arg Ser Glu Arg Ser Pro His Gly Asp Ile Ala Ile Glu
565 570 575

Phe Ser Gly Leu Arg Pro Gly Glu Lys Leu Tyr Glu Glu Leu Leu Ile
580 585 590

Gly Asp Asn Val Asn Pro Thr Asp His Pro Met Ile Met Arg Ala Asn
595 600 605

Glu Glu His Leu Ser Trp Glu Ala Phe Lys Val Val Leu Glu Gln Leu
610 615 620

Leu Ala Ala Val Glu Lys Asp Asp Tyr Ser Arg Val Arg Gln Leu Leu
625 630 635 640

Arg Glu Thr Val Ser Gly Tyr Ala Pro Asp Gly Glu Ile Val Asp Trp
645 650 655

Ile Tyr Arg Gln Arg Arg Arg Glu Pro
660 665

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 463 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Pseudomonas aeruginosa
(B) STRAIN: PA01

(vii) IMMEDIATE SOURCE:
(B) CLONE: psbN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Met Ile Asn Ser His Leu Leu Tyr Arg Leu Ser Tyr Arg Gly Thr Ala
1 5 10 15

Arg Arg Met Leu Leu Ile Lys Lys Gly Lys Pro Leu Pro Met Thr Ser
20 25 30

Pro Phe Ser Leu Gln Asp Leu Asp Asp Gly Leu Gly Asp Gly Leu Gln
35 40 45

Val Arg Phe Val Gln Arg Gly Asp Ala Asp Thr Ala Gly Ala Asp Gly
50 55 60

Val Asp Thr Glu Leu Gly Leu Gln Ala Leu Asp Leu Val Gly Gly Gln
65 70 75 80

Ala Gly Ile Gly Glu His Ala Thr Leu Ala Thr Asp Glu Thr Glu Val
85 90 95

Ala Leu Gly Ala Val Gly Cys Gln Leu Leu Asp His Arg Gln Ala His
100 105 110

Val Ala Asp Ala Val Ala His Leu Ala Gln Phe Leu Leu Pro Glu Gly
115 120 125

Pro Gln Phe Arg Ala Val Glu His Gly Gly Asp Asp Ala Gly Ala Val
130 135 140

Gly Arg Trp Val Arg Ile Val Gly Ala Asp His Pro Leu His Leu Gly
145 150 155 160

Gln His Ala Gly Arg Phe Ile Ala Ala Phe Gly His Asp Arg Glu Gly
165 170 175

Ala Asp Ala Phe Ala Ile Glu Arg Glu Gly Phe Gly Glu Arg Ala Gly
180 185 190

Asn Glu Glu Ala Gln Ala Arg Leu Gly Glu Gln Ala His Arg Gly Gly
195 200 205

Val Phe Leu Asp Ala Val Ala Glu Ala Leu Val Gly Asp Val Glu Glu
210 215 220

Arg His Val Ala Leu Gly Leu Glu His Val Gln His Leu Phe Pro Val
225 230 235 240

Val Gln Leu Glu Ile Asp Ala Gly Arg Ile Met Ala Ala Gly Val Gln
245 250 255

Asn His Asp Arg Ala Gly Arg Gln Gly Ile Gln Val Phe Gln Gln Ala
260 265 270

Gly Ala Val His Ala Ile Ala Gly Gly Val Val Ile Ala Val Val Leu
275 280 285

His Arg Glu Ala Gly Gly Phe Glu Gln Cys Ala Val Val Phe Pro Ala
290 295 300

Arg Val Ala Asp Gly His Gly Gly Val Gly Gln Gln Ala Leu Glu Glu
305 310 315 320

Val Gly Ala Glu Leu Glu Arg Ala Gly Ala Ala Asp Gly Leu Gly Arg
325 330 335

Asp His Thr Ala Gly Gly Gln Gln Leu Gly Leu Val Thr Glu Gln Gln
340 345 350

Phe Leu Tyr Ala Leu Val Val Gly Gly Asp Pro Phe Asp Arg Gln Val
355 360 365

Ala Ala Arg Arg Val Gly Leu Asp Ala Gly Leu Leu Gly Ser Leu His
370 375 380

Gly Thr Gln Gln Arg Asn Ala Pro Leu Leu Val Val Val His Ala His
385 390 395 400

Ala Gln Val Asp Leu Ala Arg Thr Gly Ile Gly Val Glu Gly Phe Val
405 410 415

Gln Ala Lys Asp Gly Ile Thr Arg Cys His Phe Asp Gly Arg Lys Gln
420 425 430

Thr His Phe Ala Ala Ala Arg Ser Val Lys Arg Gly Gly Gln Arg Asn
435 440 445

Pro Leu Cys Gly Gly Ala Lys Gly Cys Ala Asn Gly Gly Leu Leu
450 455 460

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 238 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Pseudomonas aeruginosa

(vii) IMMEDIATE SOURCE:
(B) CLONE: uvrB

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met His Ala Ala Thr Phe Arg Cys Met Leu Ser Ala Ile Ser Asp Ala
1 5 10 15

Gly Phe Ser Leu Ala Ser Gln Leu Pro Ala Arg Phe Phe Met Asp Thr
20 25 30

Phe Gln Leu Asp Ser Arg Phe Lys Pro Ala Gly Asp Gln Pro Glu Ala
35 40 45

Ile Arg Gln Met Val Glu Gly Leu Glu Ala Gly Leu Ser His Gln Thr
50 55 60

Leu Leu Gly Val Thr Gly Ser Gly Lys Thr Phe Ser Ile Ala Asn Val
65 70 75 80

Ile Ala Gln Val Gln Arg Pro Thr Leu Val Leu Ala Pro Asn Lys Thr
85 90 95

Leu Ala Ala Gln Leu Tyr Gly Glu Phe Lys Thr Phe Phe Pro His Asn
100 105 110

Ser Val Glu Tyr Phe Val Ser Tyr Tyr Asp Tyr Tyr Gln Pro Glu Ala
115 120 125

Tyr Val Pro Ser Ser Asp Thr Tyr Ile Glu Lys Asp Ser Ser Ile Asn
130 135 140

Asp His Ile Glu Gln Met Arg Leu Ser Ala Thr Lys Ala Leu Leu Glu
145 150 155 160

Arg Pro Asp Ala Ile Ile Val Ala Thr Val Ser Ser Ile Tyr Gly Leu
165 170 175

Gly Asp Pro Ala Ser Tyr Leu Lys Met Val Leu His Leu Asp Arg Gly
180 185 190

Asp Arg Ile Asp Gln Arg Glu Leu Leu Arg Arg Leu Thr Ser Leu Gln
195 200 205

Tyr Thr Arg Asn Asp Met Asp Phe Ala Arg Ala Thr Phe Arg Val Arg
210 215 220

Gly Asp Val Ile Asp Ile Phe Pro Ala Glu Ser Asp Leu Glu
225 230 235

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 303 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Pseudomonas aeruginosa
(B) STRAIN: PA01

(vii) IMMEDIATE SOURCE:
(B) CLONE: psbL

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met Met Ile Trp Met Ile Ala Cys Leu Val Val Leu Leu Phe Ser Phe
1 5 10 15

Val Ala Thr Trp Gly Leu Arg Arg Tyr Ala Leu Ala Thr Lys Leu Met
20 25 30

Asp Val Pro Asn Ala Arg Ser Ser His Ser Gln Pro Thr Pro Arg Gly
35 40 45

Gly Gly Val Ala Ile Val Leu Val Phe Leu Ala Ala Leu Val Trp Met
50 55 60

Leu Ser Ala Gly Ser Ile Ser Gly Gly Trp Gly Gly Ala Met Leu Gly
65 70 75 80

Ala Gly Ser Gly Val Ala Leu Leu Gly Phe Leu Asp Asp His Gly His
85 90 95

Ile Ala Ala Arg Trp Arg Leu Leu Gly His Phe Ser Ala Ala Ile Trp
100 105 110

Ile Leu Leu Trp Thr Gly Gly Phe Pro Pro Leu Asp Val Val Gly His
115 120 125

Ala Val Asp Leu Gly Trp Leu Gly His Val Leu Ala Val Phe Tyr Leu
130 135 140

Val Trp Val Leu Asn Leu Tyr Asn Phe Met Asp Gly Ile Asp Gly Ile
145 150 155 160

Ala Ser Val Glu Ala Ile Gly Val Cys Val Gly Gly Ala Leu Ile Tyr
165 170 175

Trp Leu Thr Gly His Val Ala Met Val Gly Ile Pro Leu Leu Leu Ala
180 185 190

Cys Ala Val Ala Gly Phe Leu Ile Trp Asn Phe Pro Pro Ala Arg Ile
195 200 205

Phe Met Gly Asp Ala Gly Ser Gly Phe Leu Gly Met Val Ile Gly Ala
210 215 220

Leu Ala Ile Gln Ala Ala Trp Thr Ala Pro Ser Leu Phe Trp Cys Trp
225 230 235 240

Leu Ile Leu Leu Gly Val Phe Ile Val Asp Ala Thr Tyr Thr Leu Ile
245 250 255

Arg Arg Ile Ala Arg Gly Glu Lys Phe Tyr Glu Ala His Arg Ser His
260 265 270

Ala Tyr Gln Phe Ala Ser Arg Arg Tyr Ala Ser His Leu Arg Val Thr
275 280 285

Leu Gly Val Leu Ala Ile Asn Thr Leu Trp Leu Leu Arg Trp His
290 295 300

WE CLAIM:

1. An isolated *P. aeruginosa* B-band gene cluster containing the following genes:*wzz, wbpA, wbpB, wbpC wbpD, wbpE, wzy, wbpF, wbpG, wbpH, wpsI, wbpJ, wbpK, wbpL, wbpM* and *wbpN* involved in the synthesis, and assembly of lipopolysaccharide in *P. aeruginosa*.

2. An isolated *P. aeruginosa* B-band gene cluster as claimed in claim 1 wherein the genes are organized as shown in Figure 1 (SEQ.ID.NO:1).

3. An isolated nucleic acid molecule encoding:

(1) (a) Wzz; (b) WbpA; (c) WbpB; (d) WbpC; (e) WbpD; (f) WbpE; (g) Wzy; (h) WbpF; (i) WbpG; (j) WbpI; (k) WbpJ; (l) WbpK; (m) WbpM; (n) WbpH; and (o) WbpN involved in *P. aeruginosa* O-antigen synthesis and assembly;

(2) UvrB involved in ultraviolet repair;

(3) HisH or HisF involved in histidine synthesis;

(4) RpsA, a 30S ribosomal subunit protein S1.

4. A nucleic acid molecule comprising nucleic acid sequences encoding two or more of the following proteins (1) (a) Wzz; (b) WbpA; (c) WbpB; (d) WbpC; (e) WbpD; (f) WbpE; (g) Wzy; (h) WbpF; (i) HisH; (j) HisF; (k) WbpG; (l) WbpI; (m) WbpJ; (n) WbpK; (o) WbpM; (p) WbpN; (q) WbpH; (r) WbpL; and (s) RpsA.

5. A recombinant molecule adapted for transformation of a host cell comprising a nucleic acid molecule as claimed in claim 3 and an expression control sequence operatively linked to the DNA segment.

6. A transformant host cell including a recombinant molecule as claimed in claim 5.

7. An isolated protein characterized in that it has part or all of the primary structural confirmation of a protein encoded by a gene of the *psb* gene cluster as claimed in claim 1.

8. A purified protein having the amino acid sequence as shown in Figure 3 or SEQ ID NO:2;, Figure 4 or SEQ ID NO:3; Figure 5 or SEQ ID NO:4; Figure 6 or SEQ ID NO:5; Figure 7 or SEQ ID NO:6; Figure 8 or SEQ ID NO:7; Figure 9 or SEQ ID NO:8; Figure 10 or SEQ ID NO:9; Figure 11 or SEQ ID NO:10; Figure 12 or SEQ ID NO:11; Figure 13 or SEQ ID NO:12; Figure 14 or SEQ ID NO:13; Figure 15 or SEQ ID NO:14; Figure 16 or SEQ ID NO:15; Figure

17 or SEQ ID NO:16; or, Figure 18 or SEQ ID NO:17; Figure 19 or SEQ.ID. No.: 18; or, Figure 20 or SEQ.ID. No.: 19.

9. A monoclonal or polyclonal antibody specific for an epitope of a purified protein as claimed in claim 8.

10. A method for detecting *P. aeruginosa* in a sample comprising contacting the sample with a monoclonal or polyclonal antibody as claimed in claim 9 which is capable of being detected after it becomes bound to protein in the sample.

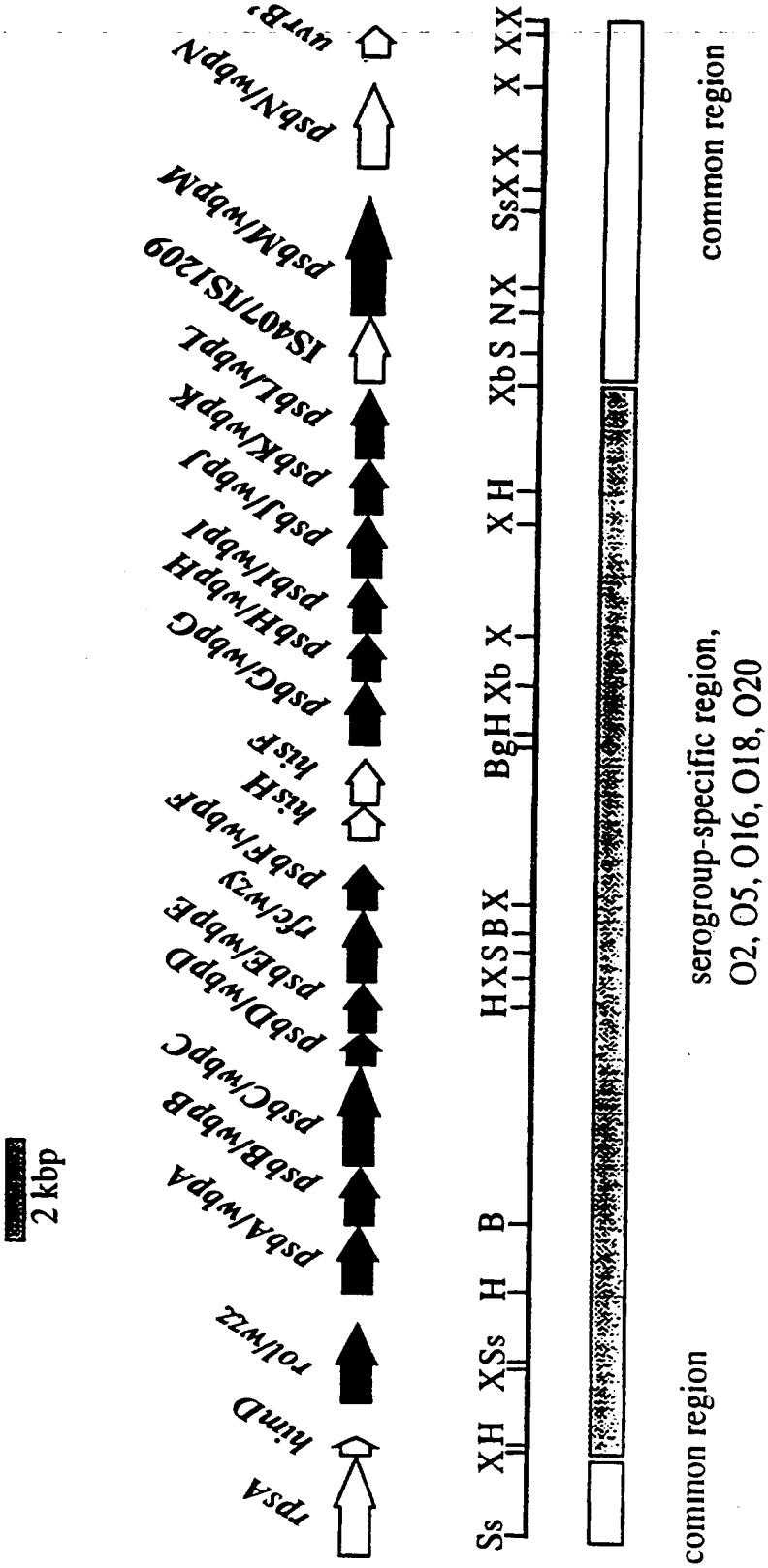
11. A method for detecting the presence of a nucleic acid molecule as claimed in claim 3 in a sample, comprising contacting the sample with a nucleotide probe capable of hybridizing with the nucleic molecule, to form a hybridization product, under conditions which permit the formation of the hybridization product, and assaying for the hybridization product.

12. A method for detecting the presence of a nucleic acid molecule as claimed in claim 3, or a predetermined oligonucleotide fragment thereof in a sample, comprising treating the sample with primers which are capable of amplifying the nucleic acid molecule or the predetermined oligonucleotide fragment thereof in a polymerase chain reaction to form amplified sequences under conditions which permit the formation of amplified sequences, and assaying for amplified sequences.

13. A kit for detecting *P. aeruginosa* by assaying for a protein involved in O-antigen synthesis or assembly in a sample comprising a monoclonal or polyclonal antibody as claimed in claim 9, reagents required for binding of the antibody to protein in the sample, and directions for its use.

14. A kit for detecting the presence of a nucleic acid molecule as claimed in claim 3 in a sample comprising a nucleotide probe capable of hybridizing with the nucleic acid molecule, reagents required for hybridization of the nucleotide probe with the nucleic acid molecule, and directions for its use.

FIGURE 1



The *Pseudomonas aeruginosa* O5 *wbp* gene cluster and flanking DNA

FIGURE 2

BASE COUNT 4990 a 5938 c 7166 g 6323 t ORIGIN

```
1     ctcgagatat tgagcagcgc atacagaact tgcggagaga atgccaaggc agacgtgaag
61    atcgtattgt tcagctcaag gaggcgttga aggtcgcagg tgcgctgaaa ttggaggagc
121   ctccactgat cagtgggcaa tcctctgagg agctctcggc tatcatgaat ggaagtctga
181   tgtatatgcg tggcagtaag gcgattatgg ccgagattca gacattggag gcgcgtagct
241   ctgatgatcc ttttattccg gcgttgcgta ctcttcagga gcagcagtta ttgctgagta
301   gcttgcgtgt taattcggag cgggtttctg tttttcgaca agacggtccg atagaaacgc
361   cggactcacc agttcgtcca aggagagcga tgattttgat ttttgggttg ataattggtg
421   gtgtgcttgg tggttttctg gcgttgtgcc ggatttttct gaagaagtat gctcgttagg
481   aaagagctag ttattgaagt ggtgatgcgt tgcacgtact ttggtcgagt aattttgtgg
541   agtaggtttt cgttgggtgg ctcgattgct gaggggtgag aacgtttcca tgcggtgttt
601   cctcagctct gtctcctgtg ccttggctcc ttgaacgcag aggttaacag ttgagctgtg
661   gttgtgggta tgtgacgtct gttgcggtgg tgtctggttc ctggtgtcgg gtgtgcgaga
721   agatgccaag ttgcctggca ggtcgttacg tgtcgtagcc gtattcgaag ctcggcaatc
781   gcggggtgat ttacaggact gtgcttaata cggcgcaggc ttggtcaggg tcgagtcggg
841   tcttcgggtg tcaactggat cgtgcgaaaa ccggtttcgt ggatgctgat aagctcggct
901   tgactggcag tccagggcgg ttaccaggtc tgtggaggcg caaaatgtat aggagcctgc
961   gtgagctggg caggctgaag gcctgctcga aagcgagtta gcattgtggt ccggaagggc
1021  atgggtggac cagagtgccg ttctgcacgg caaaagccaa cttgctcgga ggttccctag
1081  cgcctatgat tacgacgccc ttcatttttg gccattgccg ccaggtgctg tggaaagcga
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1201  tcgactgacg aatgaatcgt ggaagattta agttcccgtt gtgcggtcgc aggcgcgggc
1261  aggtaaaatt gaggtgagtt ggaaaatgat agatgttaac acagtggtag agaagttcaa
1321  aagccgacag gccttgattg gtatcgtggg tctgggttat gtcggtttac cactgatgct
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1561  gatcctttgt gtgccgacgc cgctgaacaa gtatcgcgag ccggatatga gctttgtcat
1621  caataccacc gacgcactaa aaccgtatct gcgcgtaggg caggtggttt cgctggaaag
1681  taccacctat ccgggaacta ccgaggaaga gttgttgcca cgcgtgcagg agggtggcct
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1861  agtcggcatt gccctgtatg aacaggccat cgaccgggtc gtgccggtca gttccaccaa
1921  ggccgccgag atgaccaagc tgttggagaa cattcatcgc gcggtcaata tcggtctggt
1981  caacgaaatg aagatcgttg ctgatcgcat gggtatcgac atctttgaag tggttgatgc
2041  tgcggcgacc aagccgttcg gtttcactcc ttactaccca gggccgggac tgggcgggca
2101  ctgtatcccg atcgatccct tctacctgac ttggaaggct cgcgaatacg gactgcatac
2161  ccgcttcatc gaactgtctg gtgaggtcaa ccaggccatg ccggaatacg tactgggcaa
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2281  gggtatcgct tataagaaga atgtcgacga catgcgcgag tcgccatccg tggaaatcat
2341  ggagctgatc gaagccaagg gtgggatggt cgcctatagc gatccgcatg tgccggtgtt
2401  cccgaagatg cgtgaacacc acttcgaact gagcagtgag ccgctgactg ccgaaaacct
2461  ggctaggttc gacgctgtag tgcttgcgac cgaccatgac aagtttgact atgagctgat
2521  caaggccgaa gccaagctag ttgttgacag ccgtggcaag taccgctccc cggcggcaca
2581  catcatcaag gcttgatcac ccatcccagc atgtccatcc gctcgtgcca gaaggccggg
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2701  gctacatcgc tcctcgccat atgcgcgcca tcaaagacac cggtaactgc ctggtttcgg
2761  cctatgacat caatgactcg gtcggtatta ttgatagcat ctctccccag agcgagtttt
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2881  cgctggacta cgtatcgatc tgctcgccca attacctgca ctacccgcat atcgctgcag
2941  gtctgcgctt gggttgcgac gtaatctgcg aaaagccgct tgttccaacc ccagagatgc
3001  tcgatcagtt ggctgttatc gagcgcgaaa ccgataagcg cctctacaac attctgcaac
3061  tgcgtcatca ccaggcgatc atcgcattga aggacaaggt cgcccgcgaa aaaagtccgc
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FIGURE 2 (Cont'd)

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3301  acaagacagc tggttatctg gagtacgagc aggcccgtgt gcgttggttt ctgtccgtgg
3361  atgctaacga cctgccggag tcggtcaagg gcaaaaagcc gacctatcgt tcgattaccg
3421  tcaacggtga ggaaatggag ttctctgaag gctttaccga tctacataca accagctacg
3481  aagaaattct cgctggtcgt ggttatggca tcgatgacgc tcgtcattgt gtggaaactg
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3601  tcgcggcgct tgcgcgttga ggtagaaaag gaggtggccg tcctcggtca cctgtttaca
3661  gcaggtttcc gcaggatcat tcatcagcat gtcatctagt agctctaaat tgctgaacgg
3721  tatggtcgcg gtaagttcag gcagaaacat tcggctggat gtccaggggc tgcgggctgt
3781  tgcagttctg gctgtgctag cttaccacgc caacagtgcc tggctcaggg ctgggtttgt
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FIGURE 2 (Cont'd)

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FIGURE 2 (Cont'd)

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10441 gaaaaccgtg aagttcaagg agcccaagta cgttggagac ccgatcaacg caatacgcat
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FIGURE 2 (Cont'd)

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FIGURE 2 (Cont'd)

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15541 catggctgtc ctgaagctgg cgtcggcggg ctacatctcg ctcaagtcag aaccgatctt
15601 ccgctttggc gtgagcccca acaagctatg ggattacatg ctggttgggt tgccagtcat
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15781 cgagcgtcgc acaatggggc aaagagggcg tgatgcggtc ctggagcatt atacctacga
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FIGURE 2 (Cont'd)

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19021 ctcacctcgc tagagtttgc tgcaagttgg cgaactcgcc agcagcaact gaagcaggaa
19081 aattgatgtc aaccccaggg cctactacct aggcagcgta ctaaaactgg gggcaggtca
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FIGURE 2 (Cont'd)

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FIGURE 3

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/codon_start=3
/product="Wzz (Rol)"
/db_xref="PID:g1545846"
/transl_table=11

/translation="RDIEQRIQNLRRECQGRREDRIVQLKEALKVAGALKLEEPPLIS

GQSSEELSAIMNGSLMYMRGSKAIMAEIQTLEARSSDDPFIPALRTLQEQQLLLSSLR

VNSERVSVFRQDGPIETPDSPVRPRRAMILIFGLIIGGVLGGFLALCRIFLKKYAR"

FIGURE 4

```
CDS             1286..2596
                /gene="wbpA"
                /codon_start=1
                /product="WbpA"
                /db_xref="PID:g1545847"
                /transl_table=11

/translation="MIDVNTVVEKFKSRQALIGIVGLGYVGLPLMLRYNAIGFDVLGI

DIDDVKVDKLNAGQCYIEHIPQAKIAKARASGFEATTDFSRVSECDALILCVPTPLNK

YREPDMSFVINTTDALKPYLRVGQVVSLESTTYPGTTEEELLPRVQEGGLVVGRDIYL

VYSPEREDPGNPNFETRTIPKVIGGHTPQCLEVGIALYEQAIDRVVPVSSTKAAEMTK

LLENIHRAVNIGLVNEMKIVADRMGIDIFEVVDAAATKPFGFTPYYPGPGLGGHCIPI

DPFYLTWKAREYGLHTRFIELSGEVNQAMPEYVLGKLMDGLNEAGRALKGSRVLVLGI

AYKKNVDDMRESPSVEIMELIEAKGGMVAYSDPHVPVFPKMREHHFELSSEPLTAENL

ARFDAVVLATDHDKFDYELIKAEAKLVVDSRGKYRSPAAHIIKA"
```

FIGURE 5

```
CDS             2670..3620
                /gene="wbpB"
                /codon_start=1
                /product="WbpB"
                /db_xref="PID:g1545848"
                /transl_table=11

/translation="MKNFALIGAAGYIAPRHMRAIKDTGNCLVSAYDINDSVGIIDSI

SPQSEFFTEFEFFLDHASNLKRDSATALDYVSICSPNYLHYPHIAAGLRLGCDVICEK

PLVPTPEMLDQLAVIERETDKRLYNILQLRHHQAIIALKDKVAREKSPHKYEVDLTYI

TSRGNWYLKSWKGDPRKSFGVATNIGVHFYDMLHFIFGKLQRNVVHFTSEYKTAGYLE

YEQARVRWFLSVDANDLPESVKGKKPTYRSITVNGEEMEFSEGFTDLHTTSYEEILAG

RGYGIDDARHCVETVNTIRSAVIVPASDNEGHPFVAALAR"
```

FIGURE 6

CDS 3689..5578
/gene="wbpC"
/codon_start=1
/product="WbpC"
/db_xref="PID:g1545849"
/transl_table=11

/translation="MSSSSSKLLNGMVAVSSGRNIRLDVQGLRAVAVLAVLAYHANSA
WLRAGFVGVDVFFVISGFIITALLVERGVKVDLVEFYAGRIKRIFPAYFVMLAIVCIV
STILFLPDDYVFFEKSLQSSVFFSSNHYFANFGSYFAPRAEELPLLHTCSIANEMQFY
LFYPVLFMCLPCRWRLPVFILLAILLFIWSGYCVFSGSQDAQYFALLARVPEFMSGAV
VALSLRDRELPARLAILAGLLGAALLVCSFIIIDKQHFPGFWSLLPCLGAALLIAARR
GPASLLLASRPMVWIGGISYSLYLWHWPILAFIRYYTGQYELSFVALLAFLTGSFLLA
WFSYRYIETPARKAVGLRQQALKWMLAASVVAIVVTGGAQFNVLVVAPAPIQLTRYAV
PESICHGVQVGECKRGSVNAVPRVLVIGDSHAAQLNYFFDVVGNESGVAYRVLTGSSC
VPIPAFDLERLPRWARKPCQAQIDAVAQSMLNFDKIIVAGMWQYQMQSPAFAQAMRAF
LVDTSYAGKQVALLGQIPMFESNVQRVRRFRELGLSAPLVSSSWQGANQLLRALAEGI
PNVRFMDFSSSAFFADAPYQDGELIYQDSHHLNEVGARRYGYFASRQLQRLFEQPQSS
VSLKP"

FIGURE 7

```
     CDS             5575..6066
                     /gene="wbpD"
                     /codon_start=1
                     /product="WbpD"
                     /db_xref="PID:g1545850"
                     /transl_table=11

/translation="MSYYQHPSAIVDDGAQIGSDSRVWHFVHICAGARIGAGVSLGQN

VFVGNKVVIGDRCKIQNNVSVYDNVTLEEGVFCGPSMVFTNVYNPRSLIERKDQYRNT

LVKKGATLGANCTIVCGVTIGEYAFLGAGAVINKNVPSYALMVGVPARQIGWIANSVS

SCS"
```

FIGURE 8

```
     CDS             6152..6982
                     /gene="wbpE"
                     /codon_start=1
                     /product="WbpE"
                     /db_xref="PID:g1545851"
                     /transl_table=11

/translation="MIEFIDLKNQQARIKDKIDAGIQRVLRHGQYILGPEVTELEDRL

ADFVGAKYCISCANGTDALQIVQMALGVGPGDEVITPGFTYVATAETVALLGAKPVYV

DIDPRTYNLDPQLLEAAITPRTKAIIPVSLYGQCADFDAINAIASKYGIPVIEDAAQS

FGASYKGKRSCNLSTVACTSFFPSKPLGCYGDGGAIFTNDDELATAIRQIARHGQDRR

YHHIRVGVNSRLDTLQAAILLPKLEIFEEEIALRQKVAAEYDLSLKQVGIGTPFIGSG"
```

FIGURE 9

```
CDS             7236..8552
                /gene="wzy (rfc)"
                /codon_start=1
                /product="Wzy (Rfc)"
                /db_xref="PID:g1545852"
                /transl_table=11

/translation="MYILARVDRSILLNTVLLFAFFSATVWVNNNYIYHLYDYMGSAK

KTVDFGLYPYLMVLALICALLCGGAIRRPGDLLVTLLVVILVPHSLVLNGANQYSPDA

QPWAGVPLAIAFGILIIGIVNKIRFHPLGALQRENQGRRMLVLLSVLNIVVLVFIFFK

SAGYFSFDFAGQYARRALAREVFAAGSANGYLSSIGTQAFFPVLFAWGVYRRQWFYLV

LGIVNALVLWGAFGQKYPFVVLFLIYGLMVYFRRFGQVRVSWVVCALLMLLLLGALEH

EVFGYSFLNDYFLRRAFIVPSTLLGAVDQFVSQFGSNYYRDTLLGALLGQGRTEPLSF

RLGTEIFNNPDMNANVNFFAIAYMQLGYVGVMAESMLVGGSVVLMNFLFSRYGAFMAI

PVALLFTTKILEQPLLTVMLGSGVFLILLFLALISFPLKMSLGKTL"
```

FIGURE 10

```
CDS             8549..9499
                /gene="wbpF"
                /codon_start=1
                /product="WbpF"
                /db_xref="PID:g1545853"
                /transl_table=11

/translation="MSAAFINRVARVLVGTLGAQLITIGVTLLLVRLYSPAEMGAFSV

WLSFATIFAVVVTGRYELAIFSTREEGELQAIVKLILQLTLLIFVAVAIAVVIGRHLI

ESMPVVIGEYWFALAVASLGLGINKLVLSLLTFQQSFNRLGVARVSLAACIAVAQVSA

AYLLEGVSGLIYGQLFGVVVATALAALWVGKSLILNCIETPWRMVRQVAVQYINFPKF

SLPADLVNTVASQVPVILLAAKFGGDSAGWFALTLKIMGAPISLLAASVLDVFKEQAA

RDYREFGNCRGIFLKTFRLLAVLALPPFIIFGSLASGPLG"
```

FIGURE 11

```
CDS             9831..10388
                /gene="hisH"
                /codon_start=1
                /product="HisH"
                /db_xref="PID:g1545854"
                /transl_table=11

/translation="MLKRVGAKAKASDSREDIEQAEKLILPGVGAFDAGMQTLRKSGL

VDVLTEQVMIKRKPVMGVCLGSQMLGLRSEEGAEPGLGWIDMDSVRFERRDDRKVPHM

GWNQVSPQLEHPILSGINEQSRFYFVHSYYMVPKDPDDILLSCNYGQKFTAAVARDNV

FGFQFHPEKSHKFGMQLFKNFVELV"
```

FIGURE 12

```
CDS             10388..11143
                /gene="hisF"
                /codon_start=1
                /product="HisF"
                /db_xref="PID:g1545855"
                /transl_table=11

/translation="MVRRRVIPCLLLKDRGLVKTVKFKEPKYVGDPINAIRIFNEKEV

DELILLDIDASRLNQEPNYELIAEVAGECFMPICYGGGIKTLEHAEKIFSLGVEKVSI

NTAALMDLSLIRRIADKFGSQSVVGSIDCRKGFWGGHSVFSENGTRDMKRSPLEWAQA

LEEAGVGEIFLNSIDRDGVQKGFDNALVENIASNVHVPVIACGGAGSIADLIDLFERT

CVSAVAAGSLFVFHGKHRAVLISYPDVNKLDVG"
```

FIGURE 13

```
CDS             11281..12411
                /gene="wbpG"
                /codon_start=1
                /product="WbpG"
                /db_xref="PID:g1545856"
                /transl_table=11

/translation="MKICSRCVMDTSDAEIVFDEAGVCNHCHKFDNVQSRQLFSDASG

EQRLQKIIGQIKKDGSGKDYDCIIGLSGGVDSSYLAVKVKDLGLRPLVVHVDAGWNSE

LAVSNIEKIVKYCGFDLHTHVINWEEIRDLQLAYMKAAVANQDVPQDHAFFASMYHFA

VKNNIKYILSGGNLATEAVFPDTWHGSAMDAINLKAIHKKYGERPLRDYKTISFLEYY

FWYPFVKGMRTVRPLNFMAYDKAKAETFLQETIGYRSYARKHGESIFTKLFQNYYLPT

KFGYDKRKLHYSSMILSGQMTRDEAQAKLAEPLYDADELQFDIEYFCKKMRITQAQFE

ELMNAPVHDYSEFANWDSRQRIAKKVQMIVQRALGRRINVYS"
```

FIGURE 14

```
CDS             12427..13548
                /gene="wbpH"
                /codon_start=1
                /product="WbpH"
                /db_xref="PID:g1545857"
                /transl_table=11

/translation="MTKVAHLTSVHSRYDIRIFRKQCRTLSQYGYDVYLVVADGKGDE

VKDGVRIVDVGVLSGRLNRILKTTRKIYEQALALGADVYHFHDPELIPVGLRLKKQGK

QVIFDSHEDVPKQLLSKPYMRPFLRRVVAVLFSCYEKYACPKLDAVLTATPHIREKFK

NINGNVLDINNFPMLGELDAMVPWASKKTEVCYVGGITSIRGVREVVKSLECLKSSAR

LNLVGKFSEPEIEKEVRALKGWNSVNEHGQLDREDVRRVLGDSVAGLVTFLPMPNHVD

AQPNKMFEYMSSGIPVIASNFPLWREIVEGSNCGICVDPLSPAAIAEAIDYLVSNPCE

AAALGRNGQRAVNERYNWDLEGRKLARFYSDLLSKRDSI"
```

FIGURE 15

CDS 13545..14633
/gene="wbpI"
/codon_start=1
/product="WbpI"
/db_xref="PID:g1545858"
/transl_table=11

/translation="MKILTIIGARPQFIKASVVSKAIIEQQTLSEIIVHTGQHFDANM
SEIFFEQLGIPKPDYQLDIHGGTHGQMTGRMLMEIEDVILKEKPHRVLVYGDTNSTLA
GALAASKLHVPIAHIEAGLRSFNMRMPEEINRILTDQVSDILFCPTRVAIDNLKNEGF
ERKAAKIVNVGDVMQDSALFFAQRATSPIGLASQDGFILATLHRAENTDDPVRLTSIV
EALNEIQINVAPVVLPLHPRTRGVIERLGLKLEVQVIDPVGYLEMIWLLQRSGLVLTD
SGGVQKEAFFFGKPCVTMRDQTEWVELVTCGANVLVGAARDMIVESARTSLGKTIQDD
GQLYGGGQASLGLLNILPSCDALRVEFK"

FIGURE 16

```
     CDS             14651..15892
                     /gene="wbpJ"
                     /codon_start=1
                     /product="WbpJ"
                     /db_xref="PID:g1545859"
                     /transl_table=11

/translation="MNVWYVHPYAGGPGVGRYWRPYYFSKFWNQAGHRSVIISAGYHH

LLEPDEKRSGVTCVNGAEYAYVPTLRYLGNGVGRMLSMLIFTMMLLPFCLILALKRGT

PDAIIYSSPHPFGVVSCWLAARLLGAKFVFEVRDIWPLSLVELGGLKADNPLVRVTGW

IERFSYARADKIISLLPCAEPHMADKGLPAGKFLWVPNGVDSSDISPDSAVSSSDLVR

HVQVLKEQGVFVVIYAGAHGEPNALEGLVRSAGLLRERGASIRIILVGKGECKEQLKA

IAAQDASGLVEFFDQQPKETIMAVLKLASAGYISLKSEPIFRFGVSPNKLWDYMLVGL

PVIFACKAGNDPVSDYDCGVSADPDAPEDITAAIFRLLLLSEDERRTMGQRGRDAVLE

HYTYESLALQVLNALADGRAA"
```

FIGURE 17

```
     CDS             15889..16851
                     /gene="wbpK"
                     /codon_start=1
                     /product="WbpK"
                     /db_xref="PID:g1545860"
                     /transl_table=11
/translation="MKAVMVTGASGFVGSALCCELARTGYAVIAVVRRVVERIPSVTY
IEADLTDPATFAGEFPTVDCIIHLAGRAHILTDKVADPLAAFREVNRDATVRLATRAL
EAGVKRFVFVSSIGVNGNSTRQQAFNEDSPAGPHAPYAISKYEAEQELGTLLRGKGME
LVVVRPPLIYANDAPGNFGRLLKLVASGLPLPLDGVRNARSLVSRRNIVGFLSLCAEH
PDAAGELFLVADGEDVSIAQMIEALSRGMGRRPALFTFPAVLLKLVMCLLGKASMHEQ
LCGSLQVDASKARRLLGWVPVETIGAGLQAAGREYILRQRERRK"
```

FIGURE 18

CDS 19678..21675
/gene="wbpM"
/codon_start=1
/product="WbpM"
/db_xref="PID:g1545862"
/transl_table=11

/translation="MLDNLRIKLLGLPRRYKRMLQVAADVTLVWLSLWLAFLVRLGTE
DMISPFSGHAWLFIAAPLVAIPLFIRFGMYRAVMRYLGNDALIAIAKAVTISALVLSL
LVYWYRSPPAVVPRSLVFNYWWLSMLLIGGLRLAMRQYFMGDWYSAVQSVPFLNRQDG
LPRVAIYGAGAAANQLVAALRLGRAMRPVAFIDDDKQIANRVIAGLRVYTAKHIRQMI
DETGAQEVLLAIPSATRARRREILESLEPFPLHVRSMPGFMDLTSGRVKVDDLQEVDI
ADLLGRDSVAPRKELLERCIRGQVVMVTGAGGSIGSELCRQIMSCSPSVLILFEHSEY
NLYSIHQELERRIKRESLSVNLLPILGSVRNPERLVDVMRTWKVNTVYHAAAYKHVPI
VEHNIAEGVLNNVIGTLHAVQAAVQVGVQNFVLISTDKAVRPTNVMGSTKRLAEMVLQ
ALSNESAPLLFGDRKDVHHVNKTRFTMVRFGNVLGSSGSVIPLFREQIKRGGPVTVTH
PSITRYFMTIPEAAQLVIQAGSMGQGGDVFVLDMGPPVKILELAEKMIHLSGLSVRSE
RSPHGDIAIEFSGLRPGEKLYEELLIGDNVNPTDHPMIMRANEEHLSWEAFKVVLEQL
LAAVEKDDYSRVRQLLRETVSGYAPDGEIVDWIYRQRRREP"

FIGURE 19

```
     CDS             22302..23693
                     /gene="wbpN"
                     /codon_start=1
                     /product="WbpN"
                     /db_xref="PID:g1545863"
                     /transl_table=11

/translation="MINSHLLYRLSYRGTARRMLLIKKGKPLPMTSPFSLQDLDDGLG

DGLQVRFVQRGDADTAGADGVDTELGLQALDLVGGQAGIGEHATLATDETEVALGAVG

CQLLDHRQAHVADAVAHLAQFLLPEGPQFRAVEHGGDDAGAVGRWVRIVGADHPLHLG

QHAGRFIAAFGHDREGADAFAIEREGFGERAGNEEAQARLGEQAHRGGVFLDAVAEAL

VGDVEERHVALGLEHVQHLFPVVQLEIDAGRIMAAGVQNHDRAGRQGIQVFQQAGAVH

AIAGGVVIAVVLHREAGGFEQCAVVFPARVADGHGGVGQQALEEVGAELERAGAADGL

GRDHTAGGQQLGLVTEQQFLYALVVGGDPFDRQVAARRVGLDAGLLGSLHGTQQRNAP

LLVVVHAHAQVDLARTGIGVEGFVQAKDGITRCHFDGRKQTHFAAARSVKRGGQRNPL

CGGAKGCANGGLL"
```

FIGURE 20

```
     CDS             23704..>24417
                     /gene="uvrB"
                     /codon_start=1
                     /product="UvrB"
                     /db_xref="PID:g1545864"
                     /transl_table=11

/translation="MHAATFRCMLSAISDAGFSLASQLPARFFMDTFQLDSRFKPAGD

QPEAIRQMVEGLEAGLSHQTLLGVTGSGKTFSIANVIAQVQRPTLVLAPNKTLAAQLY

GEFKTFFPHNSVEYFVSYYDYYQPEAYVPSSDTYIEKDSSINDHIEQMRLSATKALLE

RPDAIIVATVSSIYGLGDPASYLKMVLHLDRGDRIDQRELLRRLTSLQYTRNDMDFAR

ATFRVRGDVIDIFPAESDLE"
```

FIGURE 21

```
     CDS             16911..17822
                     /gene="wbpL"
                     /codon_start=1
                     /product="WbpL"
                     /db_xref="PID:g1545861"
                     /transl_table=11

/translation="MMIWMIACLVVLLFSFVATWGLRRYALATKLMDVPNARSSHSQP

TPRGGGVAIVLVFLAALVWMLSAGSISGGWGGAMLGAGSGVALLGFLDDHGHIAARWR

LLGHFSAAIWILLWTGGFPPLDVVGHAVDLGWLGHVLAVFYLVWVLNLYNFMDGIDGI

ASVEAIGVCVGGALIYWLTGHVAMVGIPLLLACAVAGFLIWNFPPARIFMGDAGSGFL

GMVIGALAIQAAWTAPSLFWCWLILLGVFIVDATYTLIRRIARGEKFYEAHRSHAYQF

ASRRYASHLRVTLGVLAINTLWLLRWH"


     source          17935..19144
                     /organism="Pseudomonas aeruginosa"
                     /insertion_seq="IS1209(PA)"
                     /strain="PAO1"
                     /serotype="05"
     misc_feature    18032..19141
                     /note="IS407"
```

FIGURE 22

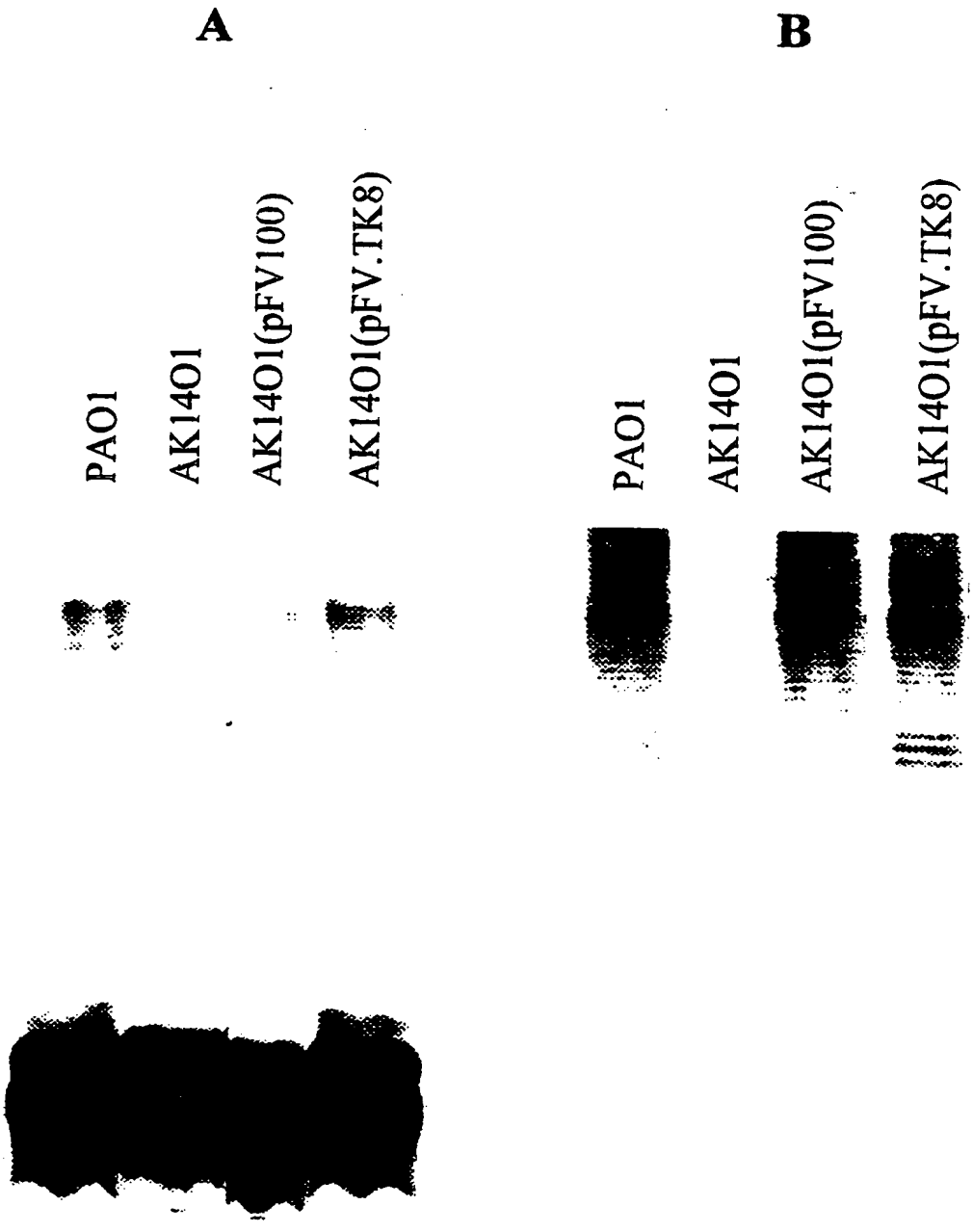

A
B
PAO1
AK1401
AK1401(pFV100)
AK1401(pFV.TK8)
PAO1
AK1401
AK1401(pFV100)
AK1401(pFV.TK8)

FIGURE 23

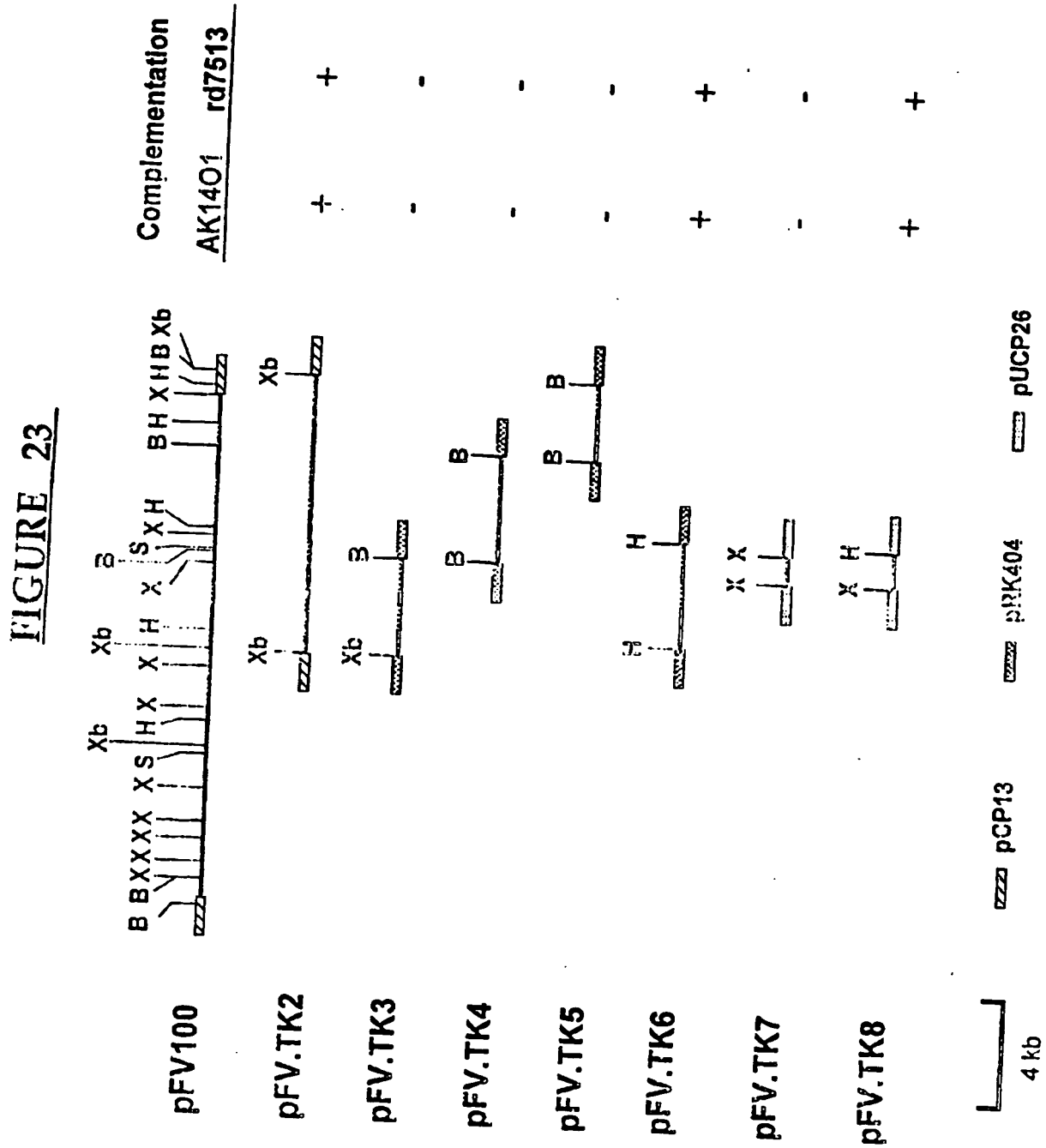

Complementation
AK1401 rd7513
pFV100
pFV.TK2
pFV.TK3
pFV.TK4
pFV.TK5
pFV.TK6
pFV.TK7
pFV.TK8
pCP13
pRK404
pUCP26
4 kb

FIGURE 24

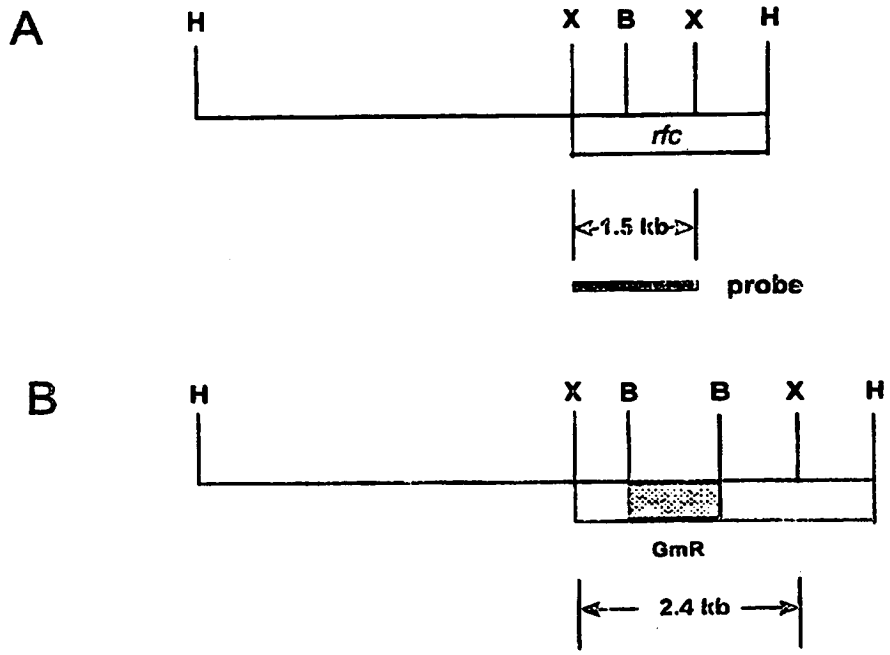


FIGURE 25

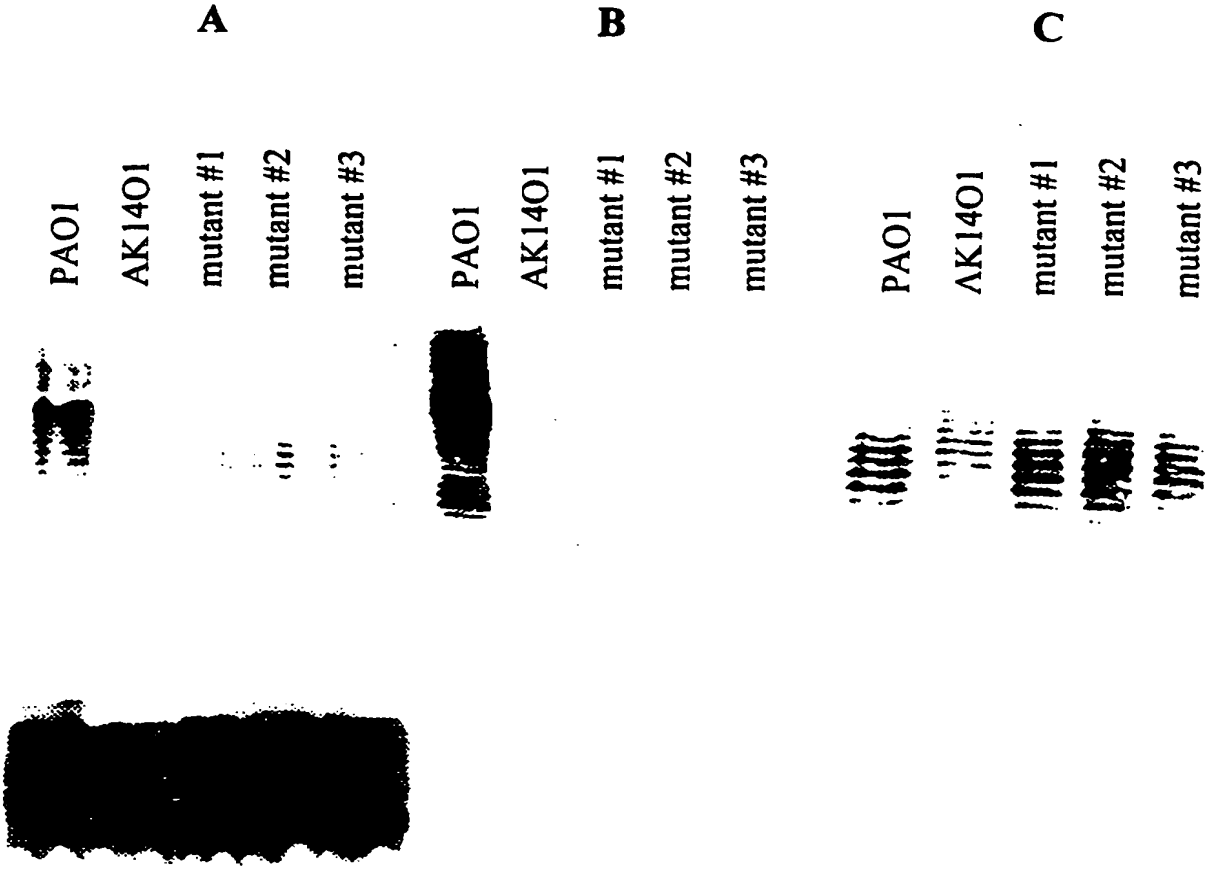

A
B
C
PAO1
AK1401
mutant #1
mutant #2
mutant #3
PAO1
AK1401
mutant #1
mutant #2
mutant #3
PAO1
AK1401
mutant #1
mutant #2
mutant #3

FIGURE 26

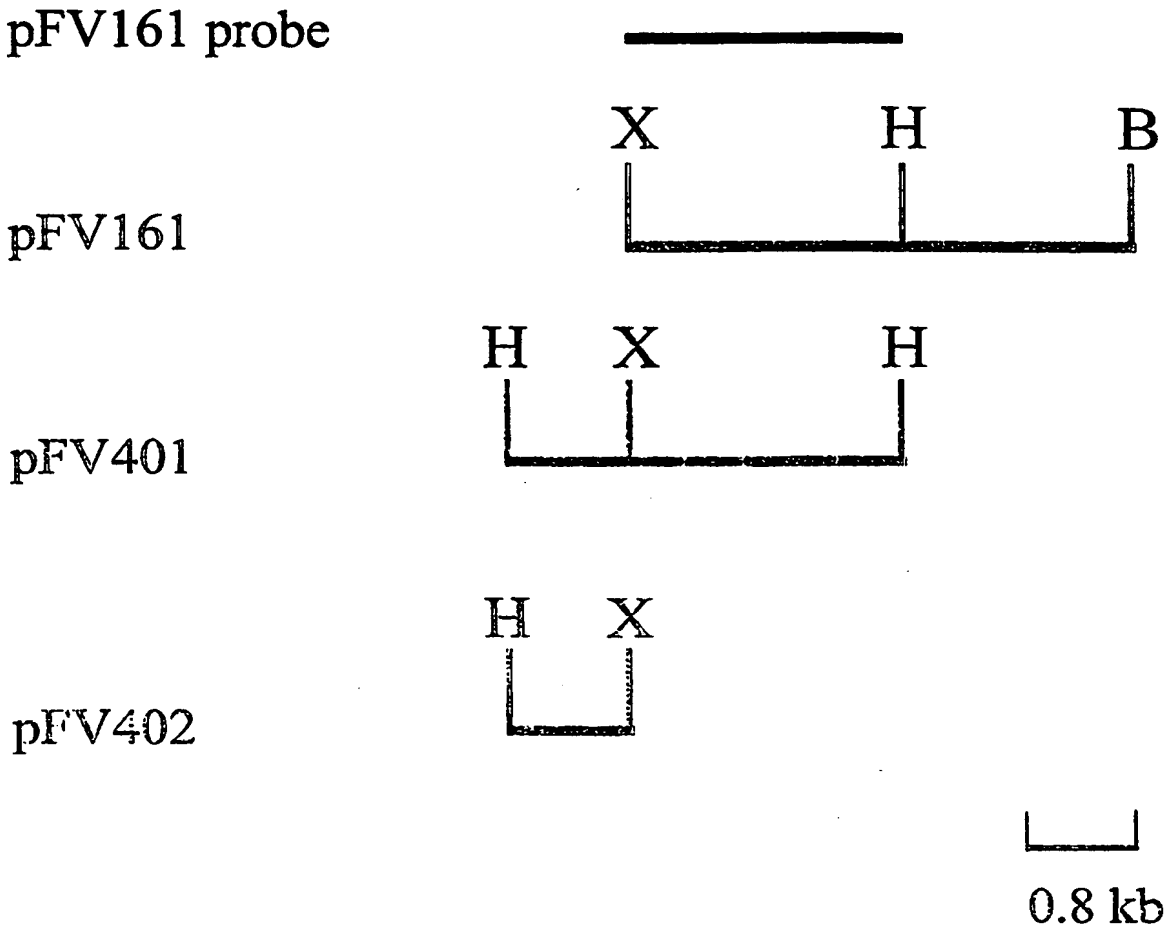


FIGURE 27

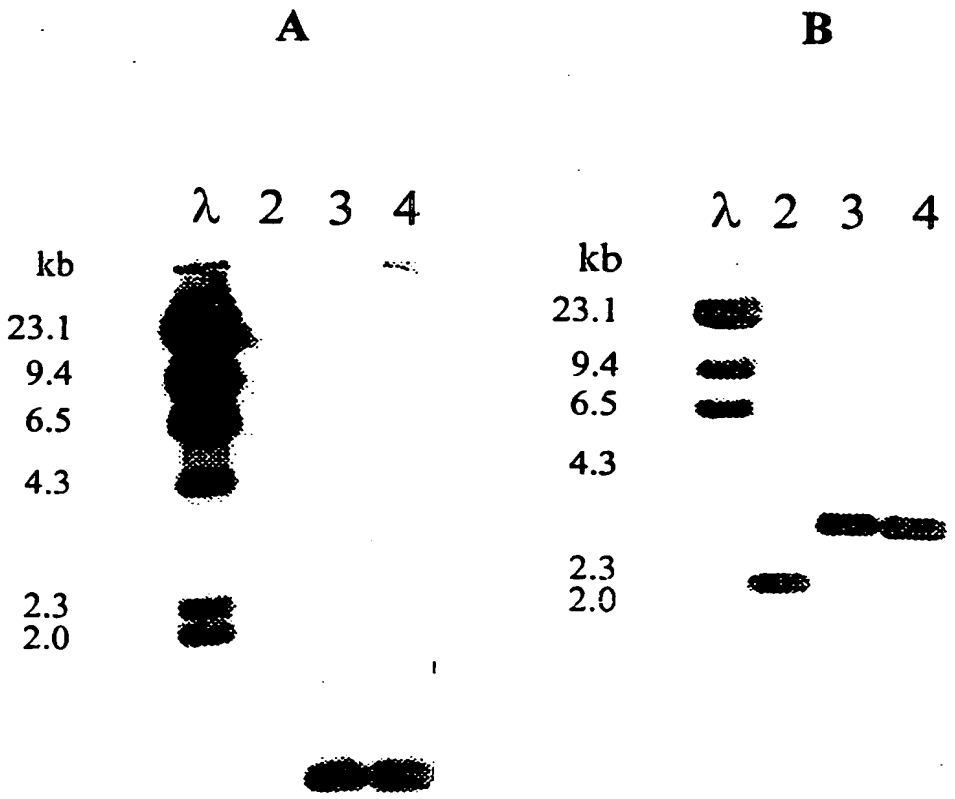


FIGURE 28

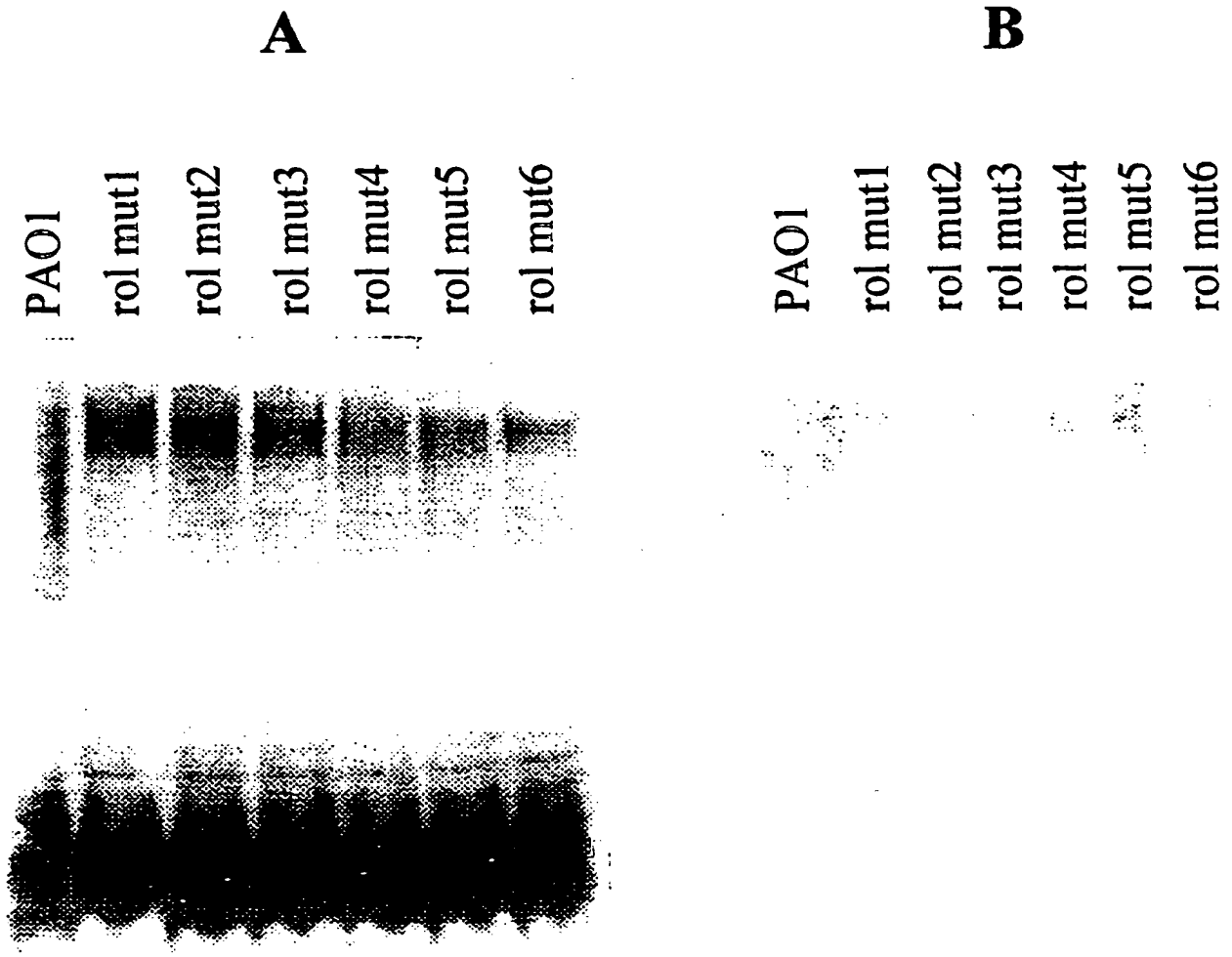

A
B
PAO1
rol mut1
rol mut2
rol mut3
rol mut4
rol mut5
rol mut6
PAO1
rol mut1
rol mut2
rol mut3
rol mut4
rol mut5
rol mut6

FIGURE 29

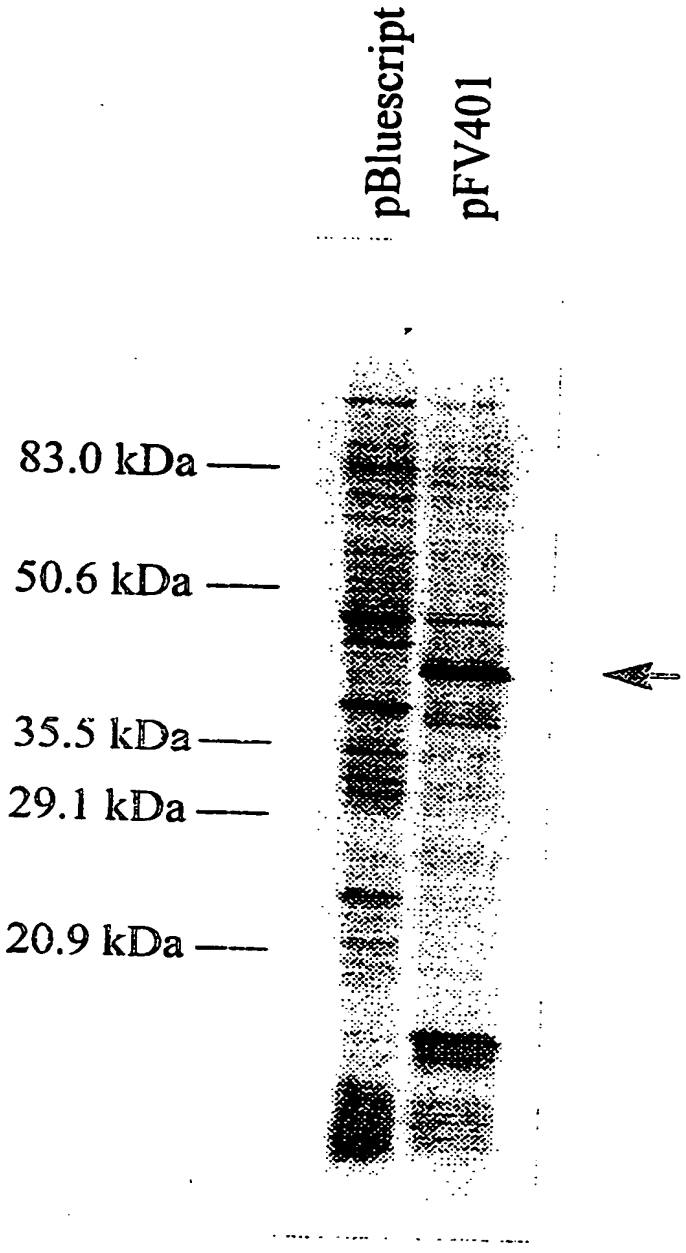
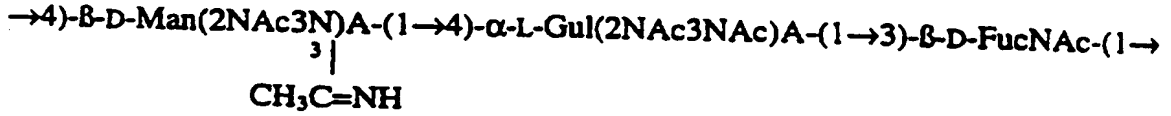
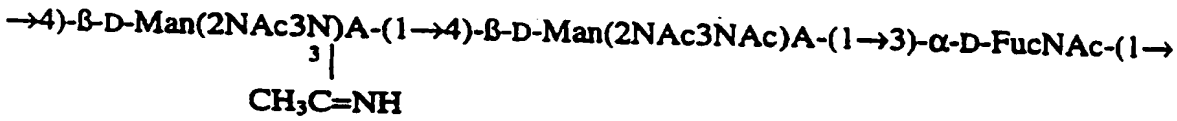


FIGURE 30

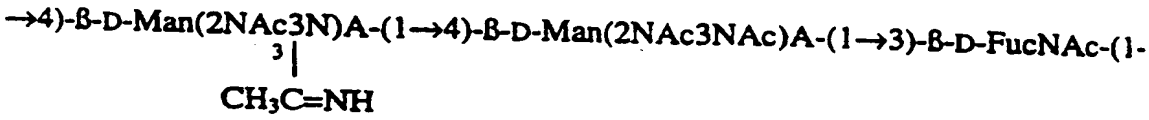
Serotype O2.



Serotype O5.



Serotype O16.



Serotype O20.

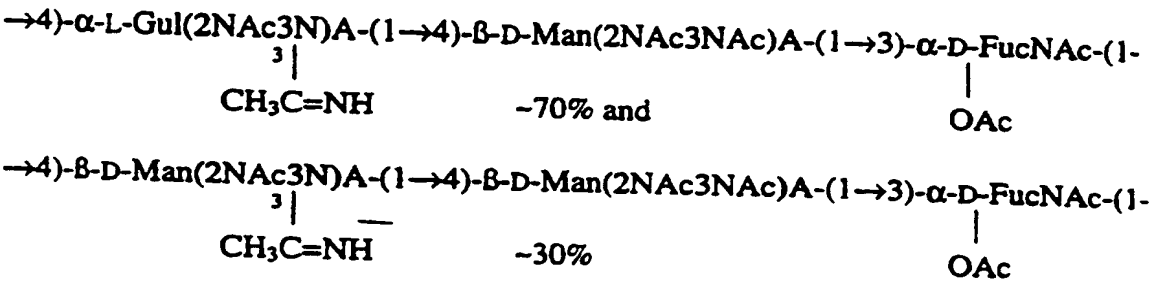


FIGURE 31

E. coli σ^{70}	c.a...t.....TTGACA..t	17bp	ggTATAATg
psbA	c.t...t.....TTGtgA..a	18bp	cgcAgAAag
hisH	t.a...t.....TTGcCc..c	16bp	gcTtTgtTg
psbG	c.a...c.....TTGgCA..g	16bp	tcaAgAtTg
IS*407*-1	c.t...g.....TTGgCA..c	17bp	agTtTgcTg
IS*407*-2	g.t...t.....TTGgCg..c	17bp	acTAagcag
IS*407*-3	t.a...g.....TTGAtg..a	17bp	acTAcctag
psbN	t.g...c.....TTGctg..a	17bp	cggATcgTc

FIGURE 32

		Spaces between RBS and first codon
	GTG ATG AAA AAA TTAA 21111111111 1111 09876543210987654321012345678901 23	
psbA	aaattGAGGTGAgttggAAAATGatagatgTTAA	8
psbB	tcatttccatAGGAcgaaccATGAAAaatttcgc	6
psbC	ctttggcAAGctgcagcgtaATGttgtgcacTTc	10
psbD	tcgagtgtGAGtctcaagccATGagttattaTcA	9
psbE	agcAAGGtGGacgtgtgaccATGattgaatTcAt	10
rfc	ctgcgttgacGAattgacggATGtatatatactt	8
psbF	atgtctttAGGAaaaactctATGagtgcggcTtt	8
hisH	tgtgccaagGGAGaTGccaaGTGatcgttgTTAt	7
hisF	aacttcgtGGAGcttgtctgATGgtccggaggcg	8
psbG	tgcttcgGGAGGTtgttGTGATGAAAgatcTgtt	4\7
psbH	cgtgatgaccggggccgctcATGactAAAgTTgc	
psbI	ctgagtaagcGAGattccatATGAAAattcTgAc	7
psbJ	taaAGGAtttatttagttccATGaacgtctggtA	13
psbK	cttgctgatgGGcgcgcagcATGAAAgctgTcAt	8
psbM	gaacggggctGATaaataggATGttggataaTtt	7
psbN	ggactcgaaccAGGgacccaATGattaacagTcA	6

FIGURE 33

Protein	position	NAD-binding domains	Reference
PsbA	17-45	LIGIVGL-GYVGLPLMLRYNAI------GGDVLGID	this study
PsbK	5-32	AVMVTGASGFVGSALCCELART-GYAVIAVVRRVVE	this study
PsbM	300-330	VVMVTGAGGSIGSELCRQIMSC-----SPSVLILFE	this study
	524-553	LVIQAGSMGQGGDVFVLDMGPP------VKILELAE	
AlgD	2-30	RISIFGL-GYVGAVCAGCLSAR------GGEVIGVD	Deretic et al., 1987
BplL	287-317	VVMVTGAGGSIGSELCRQILAL-----RPRKLVLFE	Allen and Maskell, 1996
	495-524	LVLQAGAMGESGSVFVLDMGEP------VLIRELAE	
CapD	283-316	TILVTGAGGSIGSEICRQVSKF--DPQKIILLGHGE	Lin et al., 1994
CapI	2-31	KILITGTAGFIGSHLAKKLIKQ------GGYVIGVD	Lin et al., 1994
CapL	4-32	NIAVVGL-GYVGLPVAVTFGNK-------HKVIGFD	Lin et al., 1994
CDH	12-41	CVLVTGGSGFVGANLVTELLDR------GYAVRSFD	
EpsD	11-39	TISVVGL-GYIGLPTATVLASR------QRELIGVD	Huang and Schell, 1995
ExoB	5-34	NILVVGGAGYIGSHTCLQLAAD------GYQPVVYD	Buendia et al., 1991
GalE	2-31	RVLVTGGSGVIGSKTCVQLLQN------GHDVIILD	Busby and Dreyfus, 1983
GraE	2-37	RLLVTGAAGFIGSHYVREILAGSYPESDDVHVTVVD	Bechtold et al., 1995
o355	3-33	KILITGGAGFIGSALVRYIINE-----TSDAVVVVD	Daniels et al., 1992
ORF1	9-36	KIGIIGL-GYVGLPLAVEFGKK-------VTTIGFD	Sh. sonnei; acc.#U34305
ORF7	8-35	KIAIIGL-GYVGLPLAAEFGKI-------RQVVGFD	E. coli; acc.#Z21706
ORF10	145-192	VYLIYGA-GSAGRQLAIALRNSENYKEVIMGMQVHD	Comstock et al., 1996
$RfbB_{Bc}$	2-32	KILVTGGAGFIGSAVVRHIINN-----TQDSVVNVD	Marolda and Valvano, 1995
RffD	5-33	TISVIGL-GYIGLPTAAAFASR------QKQVIGVD	Meier-Dieter et al., 1992
StrP	2-31	RILLTGHQGYLGTVMAPVLTAA------GHQVTGLD	Str. glauciens; acc.#629223
TrsG	280-310	VVMVTGAGGSIGSELCRQIIVE-----KPSLLILFD	Skurnik et al., 1995
	490-516	LVIQAGAMGQGGDVFVLDMGDP---------VKIID	
UGD	2-30	RVAIFGT-GYVGLVTGTCLAEV------GHHVICVD	Lin et al., 1995
VipA	8-35	KIAIIGL-GYVGLPLAVEFGKS-------RQVVGFD	Hashimoto et al., 1993
VipB	17-49	RWLITGVAGFIGSGLLEELLFL------NQTVIGLD	Hashimoto et al., 1993
		GxxGxxG	

FIGURE 34

```
K-tuple value   : 1
Gap penalty     : 5
Window size     : 10
Filtering level : 2.5
Open gap cost   : 10
Unit gap cost   : 10

Setting of other parameters
***************************

The alignment was done on 3 Protein sequences.
Character to show that a position in the alignment is perfectly conserved: '*'
Character to show that a position is well conserved: '.'

Alignment

PSBA        MIDVNTVVEKFKSRQALIGIVGLGYVGLPLMLRYNAIGFDVLGIDIDDVK    50
EC_RFFD     M--S------FAT----ISVIGLGYIGLPTAAAFASRQKQVIGVDINQHA    38
BS_EPSD     M--DRAIEIDFRT----ISVVGLGYIGLPTATVLASRQRELIGVDINQHA    44
                     *  .   *...****.***        ..   ...*.**..

PSBA        VDKLNAGQCYIEHIPQAKIAKARAS-GFEATTDFSRVSECDALILCVPTP    99
EC_RFFD     VDTINRGEIHIVEPDLASVVKTAVEGGFLRAS--TTPVEADAWLIAVPTP    86
BS_EPSD     VDTINQARIHIVEPDLDMLVRAAVSQGYLRAT--TEPEPADAFLIAVPTP    92
            **..*  .  .*   .  . .... .. *.  ..   .       ** .. ****

PSBA        LNKYREPDMSFVINTTDALKPYLRVGQVVSLESTTYPGTTEEELLPRVQE   149
EC_RFFD     FKGDHEPDMTYVESAARSIAPVLKKGALVILESTS-PVGSTEKMAEWLAE   135
BS_EPSD     FLEDKQPDLTYIEAAAKAIAPVLKRGDLVVLESTS-PVGATEQLSAWLSE   141
            .   ..**.... ... .. * *. *..* ****. * ...*..  .. *

PSBA        --------GGLVVGRDIYLVYSPEREDPGNPNFETRTIPKVIGGHTPQCL   191
EC_RFFD     MRPDLTFPQQVGEQADVNIAYCPERVLPGQVMVELIKNDRVIGGMTPVCS   185
BS_EPSD     QRSDLSFPHQLGEESDIRVAHCPERVLPGHVLRELVENDRIIGGMTPRCS   191
                      .  *. ....*** **.  *  .  ..*** ** *

PSBA        EVGIALYEQAIDRVVPVSSTKAAEMTKLLENIHRAVNIGLVNEMKIVADR   241
EC_RFFD     ARASELYKIFLEGECVVTNSRTAEMCKLTENSFRDVNIAFANELSLICAD   235
BS_EPSD     QAAQRLYELFVRGRCIVTDARTAEMCKLTENAFRDVNIAFANELSMICDE   241
            . .  **.  .   *.....*** ** **  *.***...**..... .

PSBA        MGIDIFEVVDAAATKPFGFTPYYPGPGLGGHCIPIDPFYLTWKAREYGLH   291
EC_RFFD     QGINVWELIRLANRHP-RVNILQPGPGVGGHCIAVDPWFIVAQNPQ---Q   281
BS_EPSD     IGVNVWELISVANRHP-RVNILQPGPGVGGHCIAVDPWFIVDAAPE---S   287
             *....*..  *. .*   .  ****.*****..**....  ...

PSBA        TRPIELSGEVNQAMPEYVLGKLMDG----LNEAGRALKGSRVLVLGIAYK   337
EC_RFFD     ARLIRTAREVNDHKPFWVIDQVKAAVADCLAATDKRASELKIACFGLAFK   331
BS_EPSD     ARLIRTAREVNDAKPHYVLDRVKQAA--------RRFKEPVIACFGLSFK   329
            .*.*  . ***. .* .*.......              .  .. .  .*...*

PSBA        KNVDDMRESPSVEIMELIEA-KGGMVAYSDPHVPVFPKMREHHFELSSEP   386
EC_RFFD     PNIDDLRESPAMEIAELIAQWHSGETLVVEPNIHQLPKKLT---GLCTLA   378
BS_EPSD     ANIDDLRESPAIEIVRTMVQQQLGTVLVVEPHIKVLPASLE---GV-ELL   375
             *.**.****..**       .  .  .*.. .*       .. .

PSBA        LTAENLARFDAVVLATDHDKFD-YELIKAEAKLVVDSRGKYRSPAAHIIK   435
EC_RFFD     QLDEALATADVLVMLVDHSQFKVINGDNVHQQYVVDAKGVWR--------   420
BS_EPSD     NAEPALSRADIVVLLVDHQKFRKLDTDRLQSRVVIDTRGMWS---AKRLA   422
              . .*.  * .*. .** .*       .  .  .  .  *.*..* ..

PSBA        A   436
EC_RFFD     -   420
BS_EPSD     A   423

Consensus length:  451
Identity   : 111 ( 24.6%)
Similarity: 154 ( 34.1%)

Dictionary of the sequences used for the alignment
**************************************************

[ 1] PSBA
     Size: 436 residues.

[ 2] EC_RFFD
     Size: 420 residues.
```

FIGURE 35

The two sequences to be aligned are:

PSBD.
Total number of residues: 163.

BP_BPLB.
Total number of residues: 191.

Comparison matrix : Structure-genetic matrix.
Open gap cost : 5
Unit gap cost : 1

The character to show that two aligned residues are identical is '|'
The character to show that two aligned residues are similar is '.'
Amino acids said to be 'similar' are: A,S,T; D,E; N,Q; R,K; I,L,M,V; F,Y,W

```
PSBD      - MSYYQHPSAIVDDGAQIGSDSRVWHFVHICAGARIGAGVSLGQNVFVGNK -50
BP_BPLB   - MTTI-HPTAIVDEGARIGANSRIWHWVHICGGAEIGAGCSLGQNVFVGNR -49

PSBD      - VVIGDRCKIQNNVSVYDNVTLEEGVFCGPSMVFTNVYNPRSLIERKDQYR -100
BP_BPLB   - VRIGDRVKIQNNVSVYDNVFLEDDVFCGPSMVFTNVYNPRAAIERKNEYR -99

PSBD      - NTLVKKGATLGANCTIVCGVTIGEYAFLGAGAVINKNVPSYALMVGVPAR -150
BP_BPLB   - DTLVRQGATLGANCTIVCGATVGRYAFVGAGAVVNKDVPDFALVVGVPAR -149

PSBD      - QIGW----------------------------IANSVSSCS  -163
BP_BPLB   - QIGWMSRHGEQLDLPLAGNGQARCPHTGDLYILENGVCRLGE -191
```

Identity : 120 (73.6%)
Similarity: 16 (9.8%)
Number of gaps inserted in PSBD: 1
Number of gaps inserted in BP_BPLB: 1

FIGURE 36

```
Setting of computation parameters
=================================

K-tuple value   : 1
Gap penalty     : 5
Window size     : 10
Filtering level: 2.5
Open gap cost   : 10
Unit gap cost   : 10

Setting of other parameters
===========================

The alignment was done on 6 Protein sequences.
Character to show that a position in the alignment is perfectly conserved:
Character to show that a position is well conserved: '.'

Alignment


PSBE        M-IEFIDLKNQQARIKDKID-AGIQRVLRHGQYILGPEVTELEDRLADFV   48
BP_BPLC     M--QFIDLKTQYQALRDTIN-PRIQAVLDHGQFIMGPEVKELEAALCAYT   47
BS_DEGT     MNVPMLDLSEQYEQLKPEIM-RVLDEVMRSSRFILGDYVKKLEADIAAYS   49
S_ERYC1     MDVPFLDLQAAYLELRSDID-QACRRVLGSGWYLHGPENEAFEAEFAAYC   49
S_DNRJ      MSTYVWQYLNEYREERADIL-DAVETVFESGQLILGTSVRSFEEEFAAYH   49
BS_SPSC     MVQKRNHFLPYSLPLIGKEEIQEVTETLESGWLSKGPKVQQFEKEFAAFV   50
                .     .       .. .  *   .*  . . .

PSBE        GAKYCISCANGTDALQIVQMALGVGPGDEVITPGFTYVATAETVALLGAK   98
BP_BPLC     GAKHCITVASGTEALLISLMALGVKAGDEVITTSFTFVATAEVIALLGAK   97
BS_DEGT     RAKHGIGCGNGSDAIHIALQAAGVGPGDEVITTAFTFFATAGSIARAGAK   99
S_ERYC1     ENAHCVTVGSGCDALELSLVALGVGQGDEVIVPSHTFIATWLGVP-VGAV   98
S_DNRJ      GLPYCTGVDNGTNALVLGLRALGIGPGDEVVTVSNTAAPTVVAIDAVGAT   99
BS_SPSC     GAKHAVAVNSCTAALFLALKAKGIGPGDEVITSPLTFSSTANTIIHTGAT  100
              . . .. .  .*. .  * *. .****..  *  .*  .  **

PSBE        PVYVDIDPRTYNLDPQLLEAAITPRTKAIIPVSLYGQCADFDAINAIASK  148
BP_BPLC     PVFVDVEPDTCNIKVSEIEAKITPRTKAIIPVSLYGQCGDMDEVNAVAAR  147
BS_DEGT     PVFVDIDPVTFNIDPAQVEAAVTEKTKAIIPVHLYGQMADMEAIAAIAKR  149
S_ERYC1     PVPVEPEGVSHTLDPALVEQAITPRTAAILPVHLYGHPADLDALRAIADR  148
S_DNRJ      PVFVDVHEENYLMDTGRLRSVIGPRTRCLLPVHLYGQSVDMTPVLELAAE  149
BS_SPSC     PVFADIDENTLNIDPVKLEAAVTPRTKAVVPVHFGGQSCDMDAILAVAQN  150
            ** . . . . .. . . .* ..** . *.  *.. . ..*

PSBE        YGIPVIEDAAQSFGASYKGKRSCNLSTVACTSFFPSKPLGCYGDGGAIFT  198
BP_BPLC     HGLPVIEDAAQSFGATYKGRKSCNLSTIGCTSFFPSKPLGCYGDGGALFT  197
BS_DEGT     HGLVVIEDAAQAIGAKYNGKCVGELGTAATYSFFPTKNLGAYGDGGMIIT  199
S_ERYC1     HGLALVEDVAQAVGARHRGHRVGAGSNAAAFSFYPGKNLGALGDGGAVVT  198
S_DNRJ      HDLKVLEDCAQAHGARRHGRLVGTQGHAAAFSFYPTKVLGAYGDGGAVVT  199
BS_SPSC     HGLFVLEDAAHAVYTTYKQRMIGSIGDATAFSFYATKNLAT-GEGGMLTT  199
            ... ..** *.. . . . . . . **...* *.  *.** . *
```

FIGURE 36 (Cont'd)

```
PSBE        NDDELATAIRQIARHG----------QDRRYHHIRV-GVNSRLDTLQAA  236
BP_BPLC     NDDELAQAMREIRVHG----------QSGRYYHARI-GVGGRMDTLQCA  235
BS_DEGT     NDDELAEKCRVIRVHG----------SKPKYYH-HVLGYNSRLDEMQAA  237
S_ERYC1     TDPALAERIRLLRNYG----------SKQKYVH-EVRGTNARLDELQAA  236
S_DNRJ      PDAEVDRRLRRLRYYG----------MGERYYVVDTPGHNSRLDEVQAE  238
BS_SPSC     DDEELADKIRVLSLHGMSKAAWNRYSSNGSWYYEVESPGYKMNMFDLQAA  249

PSBE        ILLPKLEIFEEEIALRQKVAAEY-----DLS------------------  262
BP_BPLC     VVLGKLERFDWEIAQRIKIGARYQQLLADLPGGACTVTVRPDR--DSVWA  283
BS_DEGT     ILSVKFPHLDRWTEQRRKHAATYTRLLEEAVGDLVVTPKEVDGRYH-VFH  286
S_ERYC1     VLRVKLRHLDDWNARRTTLAQHYQTELKDVPG---ITLPETHPWADSAWH  283
S_DNRJ      ILRRKLRRLDAYVEGRRAVARRYEEGLGDLDGLVLPTIAEGN---DHVYY  285
BS_SPSC     LGLHQLKRLDDMQKRREEIAGRYQTAFQQIPG-LITPFVHDDGR--HAWH  296

PSBE        ----------------------LKQV-GIGTPFI----------------  273
BP_BPLC     QFTVMVPN------REAVIAQLKEA-GIPTAVHYPRPIHAQPAYE-QYAE  325
BS_DEGT     QYTIRAPK------RDELQAFLKEQ-GIATMVYYPLPLHLQPVFA-SLGY  328
S_ERYC1     LFVLRCEN------RDHLQRHLTDA-GVQTLIHYPTPVHLSPAYA-DLGL  325
S_DNRJ      VYVVRHPE------RDRILEALTAY-DIHLNISYPWPVHTMSGFA-HLGY  327
BS_SPSC     LYVLQVDEKKAGVTRSEMITALKDEYNIGTSVHF-IPVHIHPYYQKQFGY  345

PSBE        GSG-----------------------------------------------
BP_BPLC     GAGATPVSDDLAARVMSLPMHPDLDEATQDKIVAALRQALN---  276
BS_DEGT     KEGQLPEAEKAAKEALSLPMFPELKEEQQQYVVEKIAEFYRHFA  366
S_ERYC1     PPGSFPVAESLAGEVLSLPIGPHLSREAADHVIATL----KAGA  372
S_DNRJ      GPGDLPVTERLAGEIFSLPMYPSLRPDAQEKVIDAVREVV-GSL  365
BS_SPSC     KEADFPNAMNYYKRTLSLPLYPSMSDDDVDDVIEAVRDIVKGAD  370
                                                          389
```

Consensus length: 394
Identity : 42 (10.7%)
Similarity: 83 (21.1%)

Dictionary of the sequences used for the alignment

[1] PSBE
Size: 276 residues.

[2] BP_BPLC
Size: 366 residues.

[3] BS_DEGT
Size: 372 residues.

[4] S_ERYC1
Size: 365 residues.

[5] S_DNRJ
Size: 370 residues.

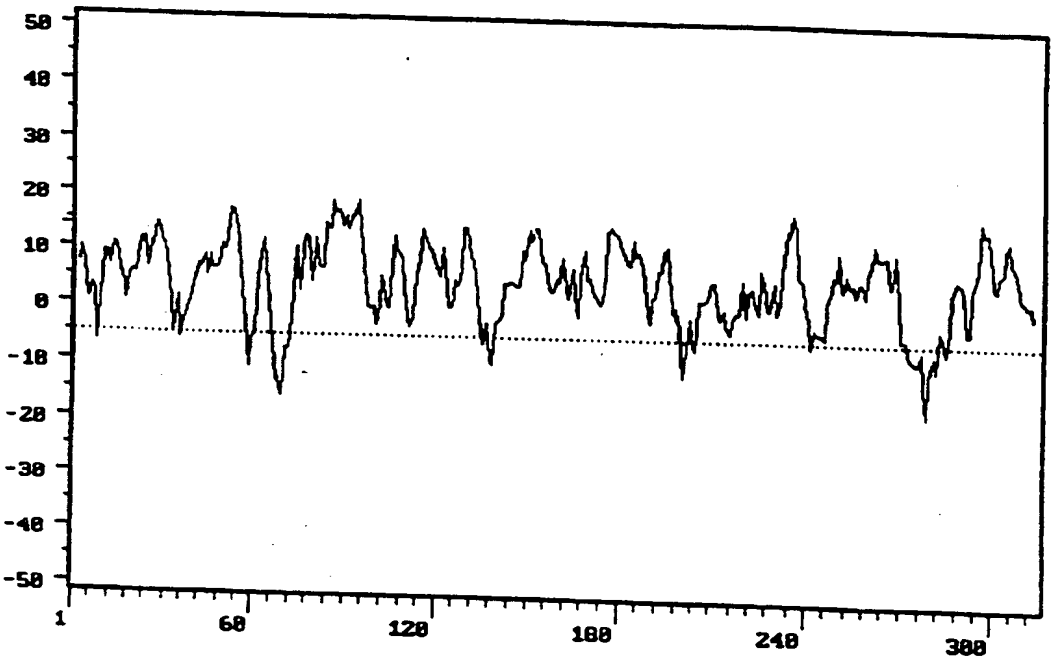
[6] BS_SPSC
Size: 389 residues.

FIGURE 37

Program SOAP.

Hydropathy index computation for sequence PSBF.

Total number of amino acids is: 316.



Hydropathic index of PSBF from amino acid 1 to amino acid 316.
Computed using an interval of 5 amino acids. (GRAVY = 10.14).

FIGURE 38

```
Setting of computation parameters
=================================

K-tuple value   : 1
Gap penalty     : 5
Window size     : 10
Filtering level : 2.5
Open gap cost   : 10
Unit gap cost   : 10

Setting of other parameters
===========================

The alignment was done on 5 Protein sequences.
Character to show that a position in the alignment is perfectly conserved:
Character to show that a position is well conserved: '.'

Alignment

PA_PSBI     M---KILTIIGARPQFIKASVVSKAIIEQQTLSEIIVHTGQHFDANMSEI    47
BP_BPLD     MPK-KILTVLGARPQFIKASVVSAAIAQHPELTEVVVHTGQHFDANMSDV    49
EC_NFRC     MK---VLTVFGTRPEAIKMAPLVHALAKDPFFEAKVCVTAQHRE--MLDQ    45
BS_ORFX     MKKLKVMTVFGTRPEAIKMAPLVLELKKYPEIDSYVTVTAQHRQ--MLDQ    48
SB_RFBC     MSK--VLFVFGTRPEAIKMAPLVIEFKNNPAIEVKVCVTGQHRE--MLDQ    46
            *     .. ..*.**. ** . . .. . . . . . *.** . * .

PA_PSBI     FFEQLGIPKPDYQLDI--HGGTHGQMTGRMLMEIEDVILKEKPHRVLVYG    95
BP_BPLD     FFDELGMQTPAHQLDI--HGGGHGDMTGRMLVALEQVMQAEKPDVVLVYG    97
EC_NFRC     VLKLFSI-VPDYDLNIMQPGQGLTEITCRILEGLKPILAEFKPDVVLVHG    94
BS_ORFX     VLDAFHI-KPDFDLNIMKERQTLAEITSNALVRLDELFKDIKPDIVLVHG    97
SB_RFBC     VLDFFEI-EPDYDLNIMKQKQSLGSITCSILTRLDEILASFMPAHIFVHG    95
              . . .  *. .*.*      . . .* . * .. ..   .*  ..*.*

PA_PSBI     DTNSTLAGALAASKLHVPIAHIEAGLRSFNM--RMPEEINRILTDQVSDI   143
BP_BPLD     DTNSTLAGALAAVKLHIPVAHVEAGLRSFNL--RMPEEVNRILTDRISRW   145
EC_NFRC     DTTTTLATSLAAFYQRIPVGHVEAGLRTGDLYSPWPEEANRTLTGHLAMY   144
BS_ORFX     DTTTTFAGSLAAFYHQIAVGHVEAGLRTGNKYSPFPEELNRQMTGAIADL   147
SB_RFBC     DTTTTFAASLAAFYQNIKVWHIEAGLRTWNMNSPFPEEGNRQLTSKLAFF   145
            **..*.*..***    .. . *.*****. .   . *** ** .*. ..

PA_PSBI     LFCPTRVAIDNLKNEGFERKAAKIVNVGDVMQDSALFFAQRATSP-IGLA   192
BP_BPLD     LFTPTDSATRHLAAEG--QSGEKVVQVGDVMYDVALHHGARVTAEGRALA   193
EC_NFRC     HFSPTETSRQNLLRE--NVADSRIFITGNTVIDALLWVRDQVMSSDKLRS   192
BS_ORFX     HFAPTGQAKDNLLKE--NKKADSIFVTGNTAIDAL---------NTTVRD   186
SB_RFBC     HAAPTLQAKDNLLRE--SVKEKNIIVTGNTVIDALLIGIKKITGSTGDVR   193
               **  .  .*  *      . ..  .*..  *

PA_PSBI     S-----QD----G---FILATLHRAENTDDPVRLTSIVEALNEIQINVA-   229
```

FIGURE 38 (Cont'd)

```
BP_BPLD     A-----HGLKPGG---YVLATIHRAENTDDAQRLTTIVRALQALAAERQ-   234
EC_NFRC     ELAANYPFIDP--DKKMILVTGHRRESFGRG--FEEICHALADIATTHQD   238
BS_ORFX     GY--SHPVLDQVGEDKMILLTAHRRENLGEP--MENMFKAIRRIVGEFED   232
SB_RFBC     EIISLKNKLNL--DKKIILVTLHRRENQGEL--LRTICDDIKQLALEHDD   239
                        .* * ** *. .   . ..  .. .  . .

PA_PSBI     -PVVLPLH--PRTRGVIERLGLKLE----VQVIDPVGYLEMIWLLQRSGL   272
BP_BPLD     --VVWPLH--PRTWGILARLGLLDELASTVTLLEPVGYLDMVQLEKYAAL   280
EC_NFRC     IQIVYPVHLNPNVREPVNR---ILGHVKNVILIDPQEYLPFVWLMNHAWL   285
BS_ORFX     VQVVYPVHLNPVVREAAHK---HFGDSDRVHLIEPLEVIDFHNFAAKSHF   279
SB_RFBC     IEIVFPVHMSPRIREVVNE---KLSGVVNIKLVEPLAYPGFIWLMNNAHF   286
              .* *.*  * ...          .  . ...* .  .  .  . .

PA_PSBI     VLTDSGGVQKEAFFFGKPCVTMRDQTEWVELVTCGANVLVGAARDMIVES   322
BP_BPLD     IATDSGGVQKEAFFHRIPCVTLRDETEWTELVDAGWNRLAPPVSSAVVAQ   330
EC_NFRC     ILTDSGGIQEEAPSLGKPVLVMRDTTERPEAVTAGTVRLVGTD-KQRIVE   334
BS_ORFX     ILTDSGGVQEEAPSLGKPVLVLRDTTERPEGVEAGTLKLAGTD-EENIYQ   328
SB_RFBC     ILSDSGGVQEEAPSLQKPVLVARDTTERPEVIENGAAMLVDPRIPNNIYS   336
            . .****.*.**     * .. ** **. * .. *   *. .      .

PA_PSBI     ARTSLGKTIQ------DDGQLYGGGQASLGLLNIL------PSCDALRVE   360
BP_BPLD     AVQDALREQP------RDVQPYGDGQAARRIVDAL------AA-------   360
EC_NFRC     EVTRLLKDENEYQAMSRAHNPYGDGQACSRILEAL-------KNNRISL-   361
BS_ORFX     LAKQLLTDPDEYKKMSQASNPYGDGEASRRIVEELLFHYGYRKEQPDSFT   376
SB_RFBC     SCKKLLSDERLYEKMSQAGNPFGDGKASKKILD-----Y-FVSLEDI---   378
                      .       . . .*.*.*   ...

PA_PSBI     FK   362
BP_BPLD     -H   362
EC_NFRC     --   376
BS_ORFX     GK   380
SB_RFBC     -K   378
```

Consensus length: 402
Identity : 71 (17.7%)
Similarity: 109 (27.1%)

Dictionary of the sequences used for the alignment

[1] PA_PSBI
Size: 362 residues.

[2] BP_BPLD
Size: 362 residues.

[3] EC_NFRC
Size: 376 residues.

[4] BS_ORFX
Size: 380 residues.

[5] SB_RFBC

FIGURE 39

```
Setting of computation parameters
=================================

K-tuple value   : 1
Gap penalty     : 5
Window size     : 10
Filtering level : 2.5
Open gap cost   : 10
Unit gap cost   : 10

Setting of other parameters
===========================

The alignment was done on 3 Protein sequences.
Character to show that a position in the alignment is perfectly conserved:
Character to show that a position is well conserved: '.'

Alignment


PA_PSBJ     MNVWYVHPYAGGPGVGRYWRPYYFSKFWNQAGHRSVIISAGYHHLLEPDE     50
BP_BPLE     ME----------------FRPYYFGREWIGHGHQVKVAASTISHIRARAP     34
YE_TRSE     M---Y------------------------EAGHNVMIISLTGETLVRPND     23
            *                                **.  .  .  .  .  ..

PA_PSBJ     KRSGVTC---VNGAEYAYVPTLRYLGNGVGRMLSMLIFTMMLLPFCLILA     97
BP_BPLE     QAGGRLTRENVDGIEYLWYATLPYQGNGARRLLNMLQFSARL--YGLRRD     82
YE_TRSE     --GIQLNELKLDKAPFSLFKGL----------------------FEVKKI     49
              .      ..   .   .*                          . .

PA_PSBJ     LKRGTPDAIIYSSPHPFGVVSCWLAARLLGAKFVFEVRDIWPLSLVELGG    147
BP_BPLE     LGGWRPDIVIASSTHPYDVLPAARLARQTGARLVFEVHDLWPLTPRLLGG    132
YE_TRSE     IKKFKPDIV---HSHMFHA--------NLFARILRVFTKIPALICTAHNT     88
            .   **  .   .*  . .            *...   .. .*     ..

PA_PSBJ     LKADNPLVRVTGWIERFSYARADKIISLLPCAEPHMADKGLPAGKFLWVP    197
BP_BPLE     FKAWHPMIASMQYAEDYAYRHADLTVSMLPCALPYMRERGLDPRRYAHVP    182
YE_TRSE     NEGSSLRMLAYKYTDKLASLSTNVSQDAV---DSFIHKGASSTGRMIAVS    135
              ..   .   .  .  .  ..  .  .  .  .  .  .  .  .  *.

PA_PSBJ     NGVDSSDISPDSAVSSSDLVR---HVQVLKEQGVFVVIYAGAHGEPNALE    244
BP_BPLE     NGVPVTEYSS-PDFDNPDYLRVRAQIRQLREQCDFVLAYAGTHGHANALD    231
YE_TRSE     NGIDASQF----DFSMDERKVKRSELGIFNDTPIILSV--GRLTEAKDYP    179
            **. .:      .  .  .      ..  ...   .:   *  .....

PA_PSBJ     GLVRSAGLLRERGASIR---IILVGKGECKEQLKAIAAQDAS-GLVEFFD    290
BP_BPLE     MLLQAMARLRDQ--PIG---LLLLGDGPDKPELKRLAGQLGL-RHIAFAD    275
YE_TRSE     NLLTAFSLLIKDNSLQSFPQLFIVGTGHLDGYLKNMSKEFGIDKYVTLFG    229
            *. . . * .          ....*.*  .  **  .. . .   ... .
```

FIGURE 39 (Cont'd)

```
PA_PSBJ     QQPKETIMAVLKLASAGYISLKSEPIFRFGVSPNKLWDYMLVGLPVIFAC   340
BP_BPLE     PVPRPAVQAVMADIDAAYIGLRRSPLFQFGVSPNKLFDYMLSACPVVQSI   325
YE_TRSE     Q--RDDILQLMCAADI-FVLSSEWEGFPLVITEA------MACKKIIVAT   270
            . . .. ...  . .. .  *.. .. .    . . .. .

PA_PSBJ     KAGNDPVSDYDCGVSADPDAPEDITAAIFRLLLLSEDERRTMGQRGRDAV   390
BP_BPLE     ESGNDIVADARCGLSVPAEDPAALAAALHGLRTLPAAERQAMGRRGRDYV   375
YE_TRSE     DAGGITEALGDCGSIVPIKDPNSLSQAINKMIKLSDNEKEILGNKARERI   320
            ..*.  .  **  . ..*.....*.  .  *...*.  .*...*. .

PA_PSBJ     LEHYTYESLALQVLNALADG---RAA--   413
BP_BPLE     LARHDYPVLAQQFLDAVQSVTPRRAASR   403
YE_TRSE     IQTNSIEKIIE--LGCLFILNLKNNC--   344
            .. .  .  *. .    ..
```

```
Consensus length:  428
Identity   : 30 ( 7%)
Similarity: 132 ( 30.8%)
```

Dictionary of the sequences used for the alignment

[1] PA_PSBJ
Size: 413 residues.

[2] BP_BPLE
Size: 403 residues.

[3] YE_TRSE
Size: 344 residues.

FIGURE 40

```
Setting of computation parameters
=================================

K-tuple value   : 1
Gap penalty     : 5
Window size     : 10
Filtering level : 2.5
Open gap cost   : 10
Unit gap cost   : 10

Setting of other parameters
===========================

The alignment was done on 3 Protein sequences.
Character to show that a position in the alignment is perfectly conserved:
Character to show that a position is well conserved: '.'

Alignment

PA_PSBL     MMIWMIACLVVLLFSFVATWGLRRYALATKLMDVPNARSSHSQPTPRGGG    50
YE_TRSF     MPTFFFLLTIFFLLSVGLTYLLRLYALKNNIIDTPNSRSSHVTPTPRGGG    50
HI_RFE      MLSIF----VTFLGAFLTLIVMRPLANWIGLVDKPNYRKRHQGTIPLIGG    46
            *  .  . . .* .     .* *   ..* ** *..*  ..*  **

PA_PSBL     VAIVLVFLAALVWMLSAGSISGGWGGAMLGAGSGVALLGFLDDHGHIAAR   100
YE_TRSF     VAIVISFLIGIILFYFLGYLPILSVVGLIVSGGVIALVGFWDDHGHIAAR   100
HI_RFE      ASLFVGNLCYYLMEWDQLRLPYLYLFSIFV----LLAIGILDDRFDISPF    92
            ... .  *   .     ..     ...     .  .*. **. .*..

PA_PSBL     WRLLGHFSAAIWILLWTGGFPPLDVVG-----HAVDLGWLGHVLAVFYLV   145
YE_TRSF     WRLLAHFSAAAFLLFCFGGFPVLNVSG-----FIIELGIFGSLFGLLFLV   145
HI_RFE      LR--AGIQAILAILMIDLGNIYLDHLGQILGPFQLTLGSIGLIITVFATI   140
             *  . . *   .*.  *   *.  *          ..** .* .....  .

PA_PSBL     WVLNLYNFMDGIDGI-ASVEAIGVCVGGALIYWLTG-HVAMVGIPLL--L   191
YE_TRSF     WMLNLYNFMDGIDGL-ASAEAVTACIGMIAIYYISGDHIELNSFLVLWLL   194
HI_RFE      AIINAFNMIDGIDGLLGGLSCVSFAAIGILMY--RDGQMDMAHWSFA--L   186
             ..* .*..*****. .. . ..      .*    . ....       *

PA_PSBL     ACAVAGFLIWNF-----PPARIFMGDAGSGFLG------MVIGALAIQAA   230
YE_TRSF     ACTVLGFLLWNF-----PPAKIFMGDAGSGFLG------LMIGSLAISAG   233
HI_RFE      IVSILPYLMLNLGIPFGPKYKVFMGDAGSTLIGFTIIWILLLSTQGKGHP   236
              ..  .*. *.     *  ..*******...*          ..... .

PA_PSBL     WTAPSLFWCWLILLGVFIVDATYTLIRRIARGEKFYEAHRSHAYQFASRR   280
YE_TRSF     WIDTRFFPCWLILLGLFIVDATWTLVRRVLGGFKVYEAHRSHGYQIASRR   283
HI_RFE      MNPVTALW----IIAIPLIDMVAIIYRRVRKGKSPFRPDRLHVHHLMVR-   281
              ..    .... ..* . .. **.  * . . ..* * ...  *
```

FIGURE 40 (Cont'd)

```
PA_PSBL     YASHLRVTLGVLAINTLWLL--R--------------------------   301
YE_TRSF     FKRHLPVTLSAIAINIIWLF--PIALLAGLNIVNPIIALIISYIPLLYI-   330
HI_RFE      --AGLTSRQAFLLITFVSAVCATIGILGEVYYVNEW-AMFVGFFILFFLY   328
                *     . . *. .

PA_PSBL     -------------------------WH    303
YE_TRSF     DYKLNAG----------------VNND   341
HI_RFE      VYSITHAWRITRWVRRMKRRAKRLKKA   355
```

Consensus length: 377
Identity : 55 (14.6%)
Similarity: 98 (26%)

Dictionary of the sequences used for the alignment

[1] PA_PSBL
Size: 303 residues.

[2] YE_TRSF
Size: 341 residues.

[3] HI_RFE
Size: 355 residues.

FIGURE 41

```
Setting of computation parameters
**********************************

K-tuple value   : 1
Gap penalty     : 5
Window size     : 10
Filtering level: 2.5
Open gap cost   : 10
Unit gap cost   : 10

Setting of other parameters
==========================

The alignment was done on 4 Protein sequences.
Character to show that a position in the alignment is perfectly conserved:
Character to show that a position is well conserved: '.'

Alignment


PSBM        MLDNLRIK--LLGLPRRYKRMLQVAADVTLVWLSLWLAFLVRLGTEDMIS     48
TRSG        MFLVF-----LLSLPRPVKRTIMLLLDTILIALAYWGAFWVRL---DVDS     42
BP_BPLL     MTLPYAIRRLFVDLPRPFKQMLAIVLDAVILLGAFHLALWLRFEL-----     45
SA_CAPD     MT----------SISAKLRFLILIIIDSFIVTFSVFLGYAI---LEPYFK     37
            *              ...  . . . * .. . .  .

PSBM        PFSG-HAWLFIAAPLVAIPLF--IRFGMYRAVMRYLGNDALIAIAKAVTI     95
TRSG        PFTSIEQWVALAA-IIPPTLFAYIKLGLYRTVLRYVSAKIVSIVLVGVVL     91
BP_BPLL     -FFLTDQYLFLSLLACAGGIAALAAFGVYLYILRYMSERVLAAILGGIVV     94
SA_CAPD     GYSIDLLVLSSVILLVSHHIFAYV-FNLYHRAWEYASVSELMSVLKAVTS     86
              .  .  .  . .  ...* * . . . ...

PSBM        SALVLSLLVYWYRSPPAVVPRSLVFNYWWLSMLLIGGLRLAMRQYFMGDW    145
TRSG        SSGLLVLGSYFL---GVYLPRTVSVMFFIFSLVLICGSRLFFRMLLN---    135
BP_BPLL     SVMVVTAGNTFLQLAT--ISRGVLVLYAALALVGLIGVRLIARKLL----    138
SA_CAPD     SIVVTLLLVSLLISESPFLR--LYFITWMMHLLLIGGSRLFWRVY---RR    131
            * .  .  .. .  . . . . * ** *

PSBM        YSAVQSVPFLNRQDGLPRVAIYGAGAAANQLVAALRLGRAMR--PVAFID    193
TRSG        YGVRGQIP----------VVIYGAGASGRQLLPALMQASEYF--PIAFVD    173
BP_BPLL     FPADHHMA-----DPRTPVLIYGAGGAGSQLAMALRTGPHYR--PVAMLD    181
SA_CAPD     Y-------FIDNAVEKKATLVVGAGQGGSVLIREMLRSQDMRMQPVLAVD    174
            .  . . . *** ... * .. . *. .*

PSBM        DDKQIANRVIAG---LRVYTAKHIRQMIDETGAQEVLLAIPSATRARRRE    240
TRSG        DNPKLHKAVIHG---VTVYPSEKLEYLIGRYGIKKVLLAMPSVSQSQRRA    220
BP_BPLL     DDKRKHRLVVNG---LRVYPPEQLPKLIDRHNIRQLLIAMPSAPPKQIRS    228
SA_CAPD     DDKNKQKMTITERVKVQGYV-EDIPELVKKFRIKKIIIAIPTLSQKRLNE    223
            *. . . . . . . * ... .. .....*.*. .. . . ..

PSBM        ILESLEPFPLHVRSMPGFMDLTSGRVKVDDLQEVDIADLLGRDSVAPRKE    290
TRSG        VVNKLENLSCEVLSIPGMSDLVEGRAQISSLKKVSIEELLGRDPVVPDEK    270
BP_BPLL     IVEAAEPYRLRIRLVPSMRELIDPTNGVR-LRDVQVEDLLGRDPVAPIDT    277
SA_CAPD     INKICNIEGVELFKMPNIEDVLSGELEVNNLKKVEVEDLLGRDPVELDMA    273
            . . .  . .*.. .. .  . *..* ...***** .*
```

FIGURE 41 (Cont'd)

```
PSBM        LLERCIRGQVVMVTGAGGSIGSELCRQIMSCSPSVLILFEHSEYNLYSIH   340
TRSG        LLAKNITGKVVMVTGAGGSIGSELCRQIIVEKPSLLILFDISEFSLYSIE   320
BP_BPLL     LLGRCVTDRVVMVTGAGGSIGSELCRQILALRPRKLVLFEIAEPALYAIE   327
SA_CAPD     LISRELTNKTILVTGAGGSIGSEICRQVSKFDPQKIILLGHGENSIYSIH   323
            *... . .....**********.***.    *  ..*.. .* ..*.*.

PSBM        QELERRIKRESLSVNLLPILGSVRNPERLVDVMRTWKVNTVYHAAAYKHV   390
TRSG        NEMAAICKKNKIETEFVALLGSVQSEKRLVQIMSNFHVNTVYHAAAYKHV   370
BP_BPLL     QDLRQRIGERNIEIA--GVLGSVRDAAHCLAQLQEHGVQTIYHAAAYKHV   375
SA_CAPD     QELSKTYGNR---IEFVPVIADVQNKTRILEVMNEFKPYAVYHAAAHKHV   370
            ...          ..   ....*..  . .. . .    ..*****.***

PSBM        PIVEHNIAEGVLNNVIGTLHAVQAAVQVGVQNFVLISTDKAVRPTNVMGS   440
TRSG        PLVENNVIEGVRNNIFGTLYCAKAAIKSGVEKFVLISTDKAVRPTNTMGA   420
BP_BPLL     PIVEHNVSEGIRTNAFGTLNMAETAIQAGVLDFVLISTDKAVRPTNVMGA   425
SA_CAPD     PLMEYNPHEAIRNNILGTKNVAESAKEGEVSKFVMISTDKAVNPSNVMGA   420
            *..* *  *.. .* .**   ...* . .* .**.*******.*.*.**.

PSBM        TKRLAEMVLQALSNESAPLLFGDRKDVHHVNKTRFTMVRFGNVLGSSGSV   490
TRSG        TKRMAELVLQALSTEQ-------------NKTKFCMVRFGNVLGSSGSV   456
BP_BPLL     SKRLAELILQA--------------HAQIQDKTRFSMVRFGNVLGSSGSV   461
SA_CAPD     TKRIAEMVIQSLNEDNS-------------KTSFVAVRFGNVLGSRGSV   456
            .**.**...*.                  **.*  *********.***

PSBM        IPLFREQIKRGGPVTVTHPSITRYFMTIPEAAQLVIQAGSMGQGGDVFVL   540
TRSG        VPLFKKQIAEGGPITLTHKDIIRYFMTIPEAAQLVIQAGAMGQGGDVFVL   506
BP_BPLL     VPLFRRQILEGGPITLTHPEITRYFMTIPEAAQLVLQAGAMGESGSVFVL   511
SA_CAPD     IPLFKNQIESGGPVTVTHPEMTRYFMTIPEASRLVLQAGALAQGGEVFVL   506
            .***. **  ***.*.** ...*********..**.***.....*.****

PSBM        DMGPPVKILELAEKMIHLSGLSVRSERSPHGDIAIEFSGLRPGEKLYEEL   590
TRSG        DMGDPVKIIDLAKRMINLSGLSIKSEENLDGDIAIEISGLRPGEKLYEEL   556
BP_BPLL     DMGEPVLIRELAERMVRLYGLTVKNSDQPDGDIEIRITGLRPGEKLYEEL   561
SA_CAPD     DMGKPVKIVDLAKNLIRLSG-------KKEEDIGIEFSGIRPGEKLYEEL   549
            *** ** * .**.....* *          ..**.* ..*.**********

PSBM        LIGDNVNPTDHPMIMRANEEHLSWEAFKVVLEQLLAAVEKDDYSRVRQLL   640
TRSG        LIGDSVQHTYHPRIMTATEIMLEWDDLNILLNKIETACNDFNYECIRSLL   606
BP_BPLL     LIGEDSRETLHPRIMRATEYSLPYETLMGQLRMLDRSLQMCSPRQAAELL   611
SA_CAPD     LNKNEIHPQ-----------------QVYEKIYRGKVDHYIKTEVDLIV   581
            *  .. .                                        ..

PSBM        RETVSGYAPDGEIVDWIYRQRRRE-------P   665
TRSG        LEAPTGFQPTDGICDVVWQKTHSENAKNVIVH   638
BP_BPLL     GQIVREYAS-------------------VTYA   624
SA_CAPD     EDLINNFS--------------KEKLLKIANR   599
              .   ..
```

```
Consensus length:  682
Identity   : 154 ( 22.6%)
Similarity: 185 ( 27.1%)
```

Dictionary of the sequences used f r the alignment

[1] PSBM
Size: 665 residues.

[2] TRSG
Size: 638 residues.

[3] BP_BPLL
Size: 624 residues.

[4] SA_CAPD
Size: 599 residues.

FIGURE 42

Entire sequence of *rol* gene:

ATGACTGACGAAATACAAAAGCACGGCGGTGTAGCTGGCGATATCGATCTGGTTGAGCTGGTTCGAGGA
TTATGGGAGGAGAAGTGGATAGTTCTTATATTTTCTTTGCTAGGTATTTTGTTTGCAGCTATCTACGCT
TTTCTCAGTACTCCTGTCTATGAGGCCCGCATAGCGATTTTGCCTCCGTCGTTGAGTGATGTGGCAGGT
TTCAATCAGGGACGTACCAGGGAAACCGGGCTTGGTCCCTTCAAGGTCCAGGATGTGTACTCTGTTTTT
GTTCGCAACCTGCAGGCTGATGGAACTCGTCATCGTTTTTTCAATGAGACCTATTTGCCTTCTTTGGAT
GAAGAGCTTCGTTCGGTTTCGCGTGATGCGCTCTATAAAAGGTTCACTGATCAGATAAGTATTAGTTTG
CCGGGGAAAGACTTTCCGGGTCGTTATCTTGTTGCGATTGAACAGGAGGATCCGGAGCGTGCGGCGAGT
TGGGTTCGTCGGTATATAGCTGATGCGGCCGAGATTTCTATTCAGGAAATGTTGAACAATGCGCATCGC
GAGATTGAGGTCAAGGCTCGAGATATTGAGCAGCGCATACAGAACTTGCGGAGAGAATGCCAAGGCAGA
CGTGAAGATCGTATTGTTCAGCTCAAGGAGGCGTTGAAGGTCGCAGGTGCGCTGAAATTGGAGGAGCCT
CCACTGATCAGTGGGCAATCCTCTGAGGAGCTCTCGGCTATCATGAATGGAAGTCTGATGTATATGCGT
GGCAGTAAGGCGATTATGGCCGAGATTCAGACATTGGAGGCGCGTAGCTCTGATGATCCTTTTATTCCG
GCGTTGCGTACTCTTCAGGAGCAGCAGTTATTGCTGAGTAGCTTGCGTGTTAATTCGGAGCGGGTTTCT
GTTTTTCGACAAGACGGTCCGATAGAAACGCCGGACTCACCAGTTCGTCCAAGGAGAGCGATGATTTTG
ATTTTTGGGTTGATAATTGGTGGTGTGCTTGGTGGTTTTCTGGCGTTGTGCCGGATTTTTTTGAAGAAG
TATGCTCGTTAG

FIGURE 43

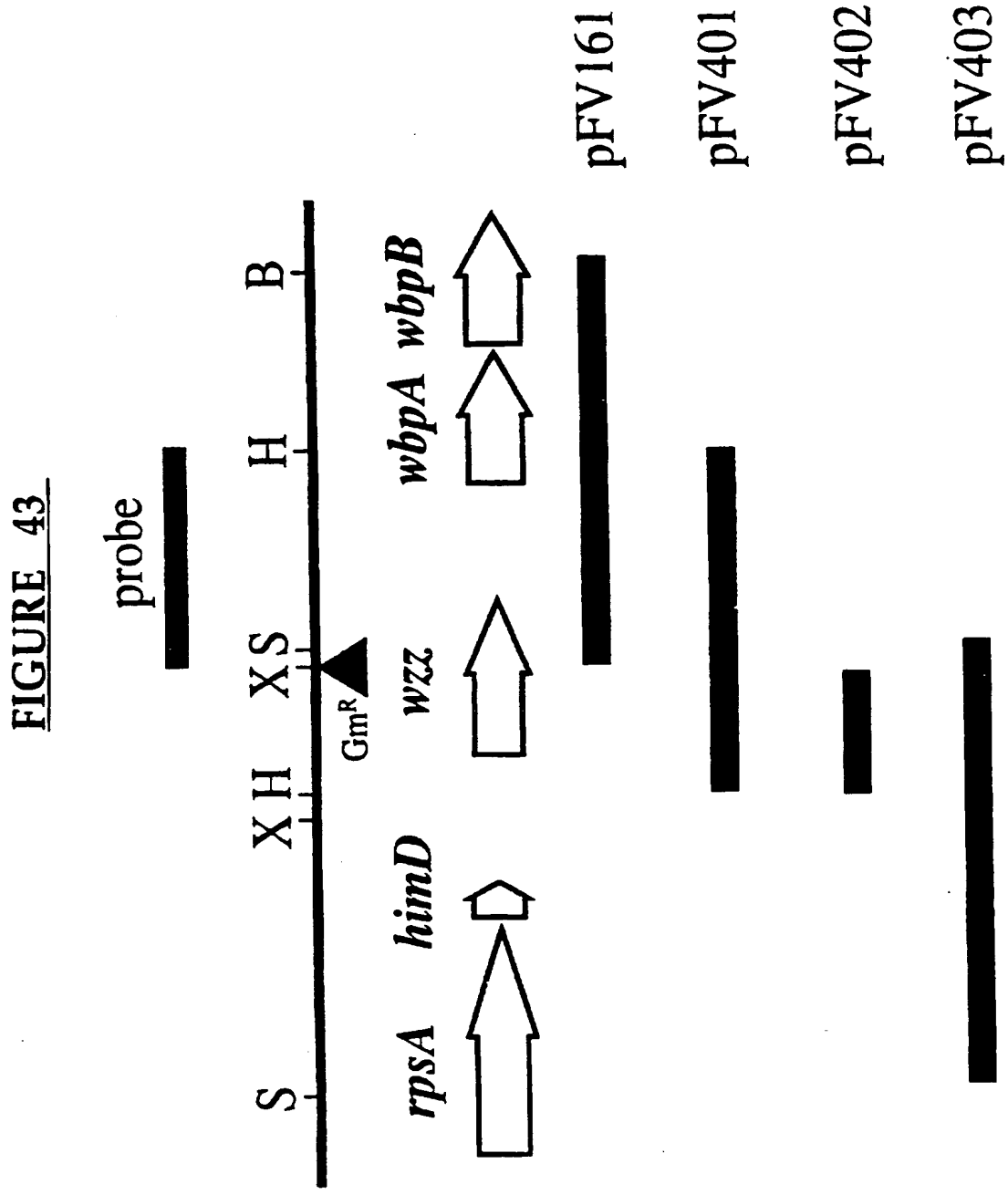

probe
S
X
H
X
S
B
H
rpsA
himD
wzz
wbpA
wbpB
GmR
pFV161
pFV401
pFV402
pFV403

FIGURE 44

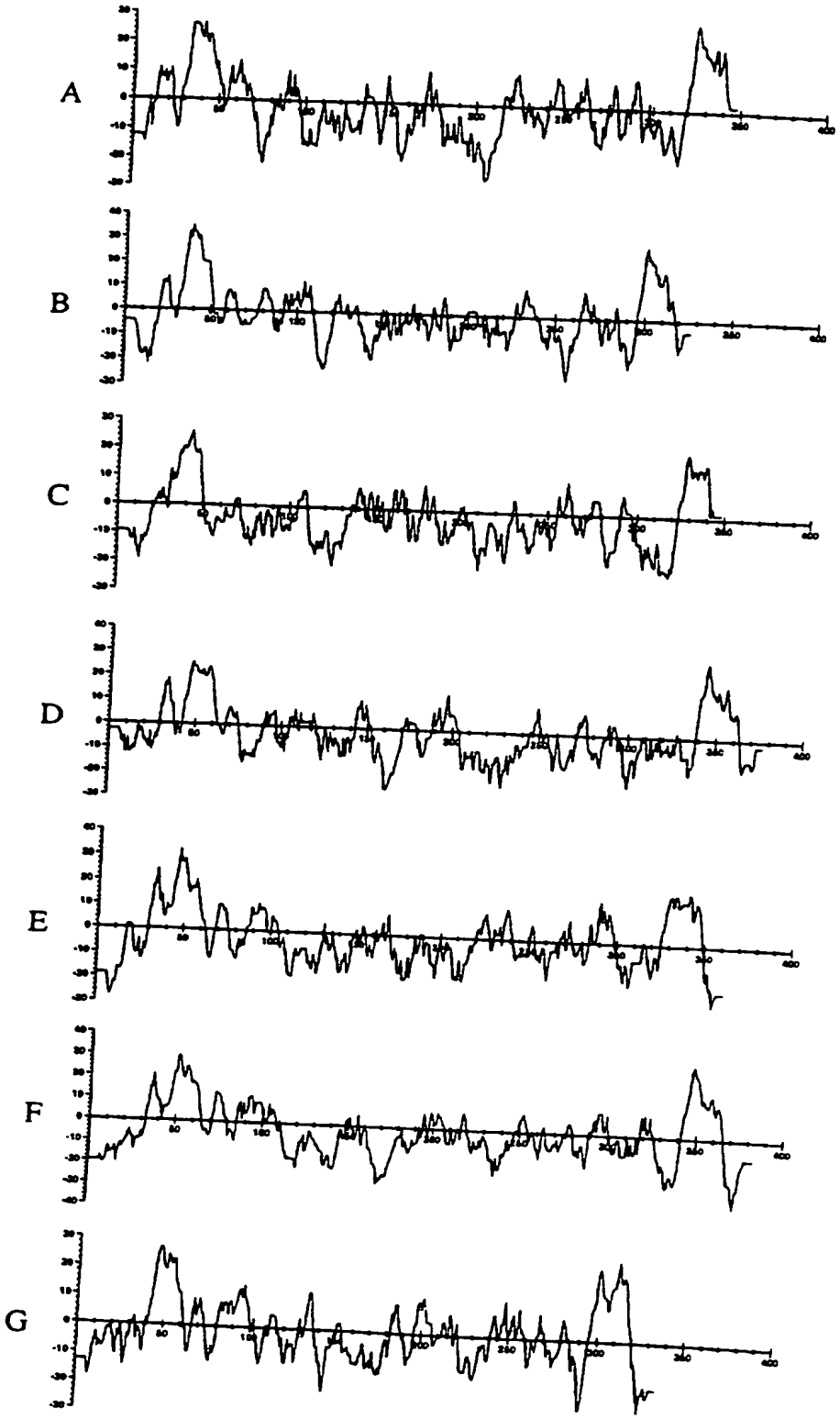
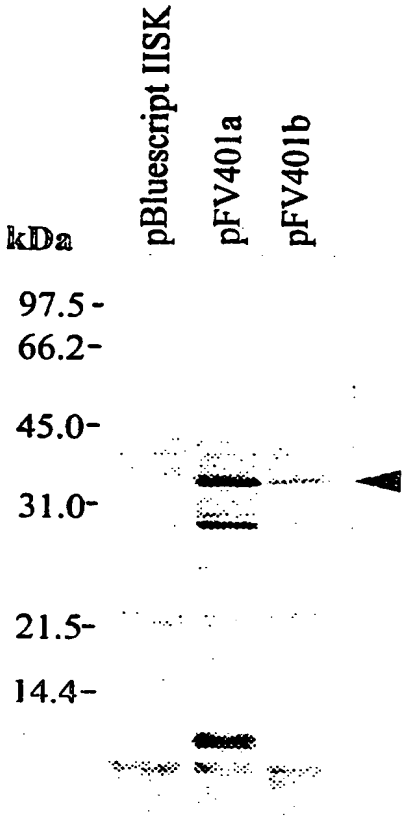


FIGURE 45


pBluescript IISK
pFV401a
pFV401b
kDa
97.5-
66.2-
45.0-
31.0-
21.5-
14.4-

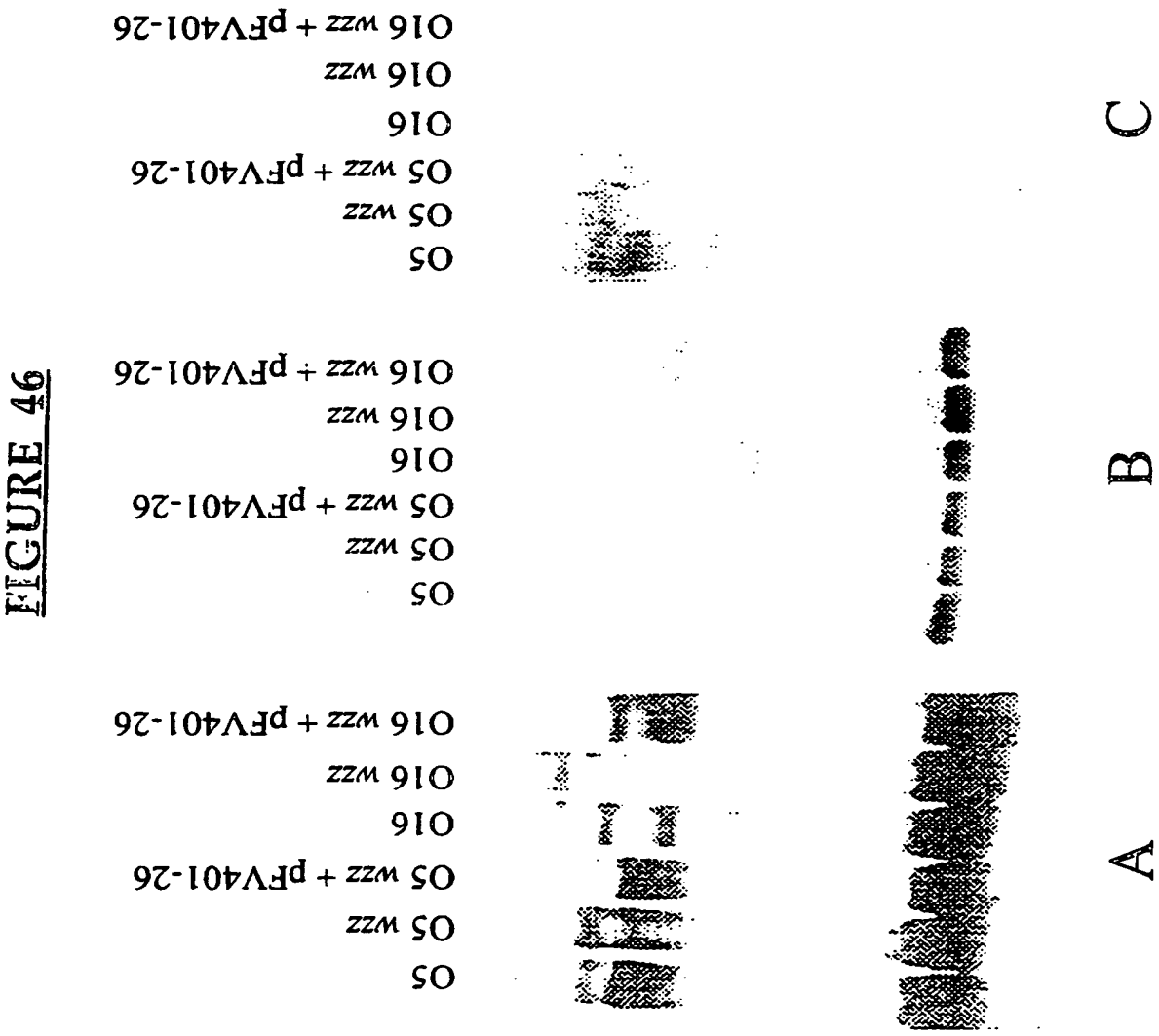

O5
O5 wzz
O5 wzz + pFV401-26
O16
O16 wzz
O16 wzz + pFV401-26
A
O5
O5 wzz
O5 wzz + pFV401-26
O16
O16 wzz
O16 wzz + pFV401-26
B
O5
O5 wzz
O5 wzz + pFV401-26
O16
O16 wzz
O16 wzz + pFV401-26
C

FIGURE 46

FIGURE 47

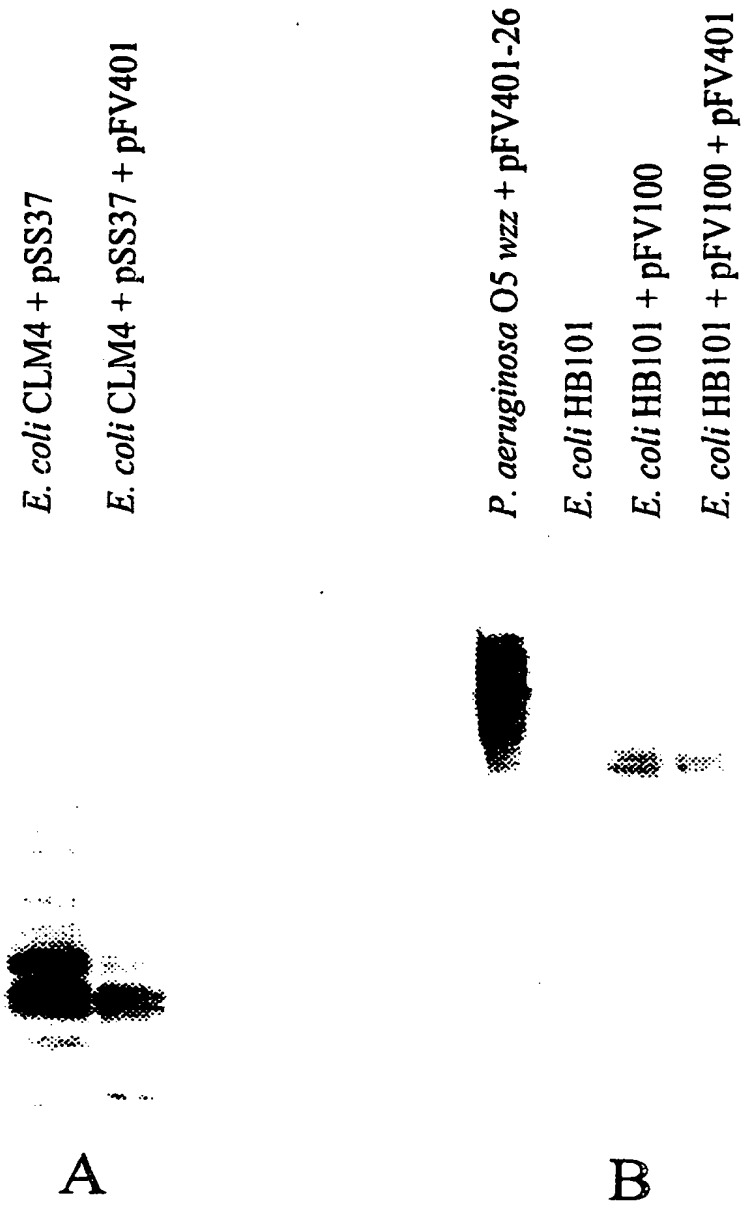

E. coli CLM4 + pSS37
E. coli CLM4 + pSS37 + pFV401
P. aeruginosa O5 wzz + pFV401-26
E. coli HB101
E. coli HB101 + pFV100
E. coli HB101 + pFV100 + pFV401
A
B

FIGURE 48

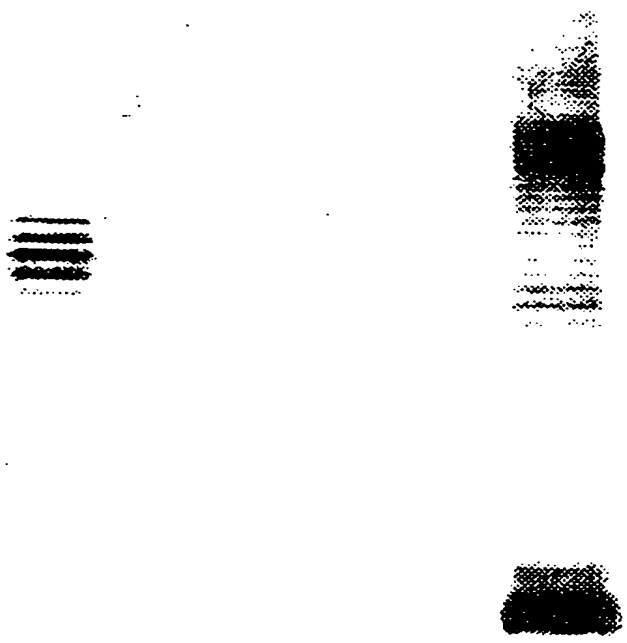


FIGURE 49

GAGCTCGAGTTCAAGGTCATCAAGCTCGACCAGAAGCGCAACAACGTTGTCGTTCCCGCCGCAGCGTCC
GGAAGCCGAGAACAGCGCCGAGCGTGAAGCTCTGCTGGAATCGCTGCAGGAAGGCCAGCAGGTCAAAGGT
TCGTCAAGAAACCTCACCGACTACGGCGCATTCGTGGACCTGGGCGGCGTAGACGGCCTGCTACACATCAC
GACATGGCCTGGAAGCGCATCAAGCATCCGTCCGAGATCGTCAACGTTGGCGACGAGATCGACGTCAAGG
CCTGAAGTTCGACCGCGAGCGCAACCGTGTATCCCTGGGCCTGAAGCAACTGGGCGAAGACCCGTGGGTT
CCATCAAGGCGCGTTACCCGGAAGTACCGCGTCATGGCCGCGTCACCAACCTCACCGACTACGGCTGCTT
GCCGAACTGGAAGAGGCGTGGAAGCTGGTACACGTCTCCGAAATGGACTGGACCAACAAGAACATCCATC
GTCGAAAGTCGTCCAGGTTGGCGATGAAGTGGAAGTTCAGGTTCTGGACATCGACGAAGAGCGTCGTCGT
TCTCCCTGGGTATCAAGCAGTGCAAATCCAACCCGTGGGAAGACTTCTCCAGCCAGTTCAACAAGGGTGA
CGTATCTCCGGTACCATCAAGTCGATCACCGACTTCGGTATCTTCATCGGTCTGGACGGCGGCATCGACG
CCTGGTCCACCCTGTCCGACATCTCCTGGAACGAAGTCGGCGAAGAAGCCGTACGTTCGCTTCAAGAAGGGC
ACGAGCTGGAAACCGTCATCCTGTCGGTCGATCCGGAGGCGCGAGCGCATCTCCCTGGGCATCAAGGCAGCT
GAAGACGATCCGTTCTCCAACTACCGCGTCCTGCACGAGAAAGCAGCATCGTCCGCGGTACCGTGAAGGAA
TCAACGCCCA

FIGURE 50

AAAAATCGAAGTATCCTGAAGGCTTCCGAAATCAGCCGTGACCGCGTCGAAGACGCGCGCAAGTCCTGAA
GAAGGGGAGGAAGTCGAAGCCAAGATTATCAGCATCGACCGCAAGAGCGGGGTCATCAGCTTTTCCGTCAA
TCCAAGGACGTCGACGACGAGAAGGACGCAATGAAAGAACTGCGTAAGCAGGAAGTAGAAAGCGCTGGTC
GACCACCATCGGTGATCTCGATCCGTGCTCAGATGGAGAATCAGGGCTAAGTCTCTGATCCATCATGAAAA
GGGCGGCCTAGGCCGCCCTTTTTCGTTTTCCCCTTCTTGGACCTGTTCAAAGACTGATCAGCATGCTAAAA
GAGACCTGAGCTGATCTAGCCGCTTGAAAAAGAAGGGAAAACCATGACCAAGTCGGAGTTGATCGAACGG
TCGTTACCCATCAGGGGCAACTGTCCGCGAAGGATGTCGAGTTGGCAATCAAGACCATGCTGGAGCAAAT
TCCCAGGCCCTGGCGACCGGGACCGGATCGAGATCCGTGGCTTCGGCAGCTTTTCCTTGCATTACCGCGC
CCGCGCGTCGTTCGTTCGCAACCCCAAGACCGGGGAGTCGGTACGTTCGACGGCAAGTTCGTGGCCGCA
GCCGGGCAAGGAGTTGCGGGATCGGTCAACGAGCCGGAGTGATTTCTGCCTTGTTCAGATGTTGGAGTT
CCATGCTTTGGGTCAAGCGTACGTTAATGGCGGTGGGGCTGTTAGTTGTCGCCCTTTTCATGATTGTGGT
GCTTTGGAGAACCGGCAAAGCGTCAGCTTTGAACTCTTTGGTCTTGCCACGCCAGATTTACCTGTGGTCC
TTATGTTGCGTTAGCATTTATTGCTGGCGGTATTATTGGTATGTTCAGCGTGCCTCTTCTGGCTCGT
CCAAAAGTGCGTCTCAGATCTGCAAGATCTGATCTGTTTCGTACTCGAAAAAGAACTCGCAGTATCTCAGTC
ACCGCCCTGCGGTGAGGTCTGCTCGAGTCCCTGCCTGTTCTTTGTGGGCTCGAGTGCTATTCGCATCTAG
TGACAACACAATGCTTGGAAGGTCGGGTGGGATGGGTGTCCTGTTAGAGGGGGTGCTGAGTTACCATGTC
TACTGGTTTGGCTGGAGTCTGTAGATGGAGTCTGTAGATGGAGGCTTGGTTCATGGCATCGTGTCGCTCC
GCTTGGTCTTCGCCAAAGGTCAAGCTT

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60/039,473	27 February 1997 (27.02.97)	US

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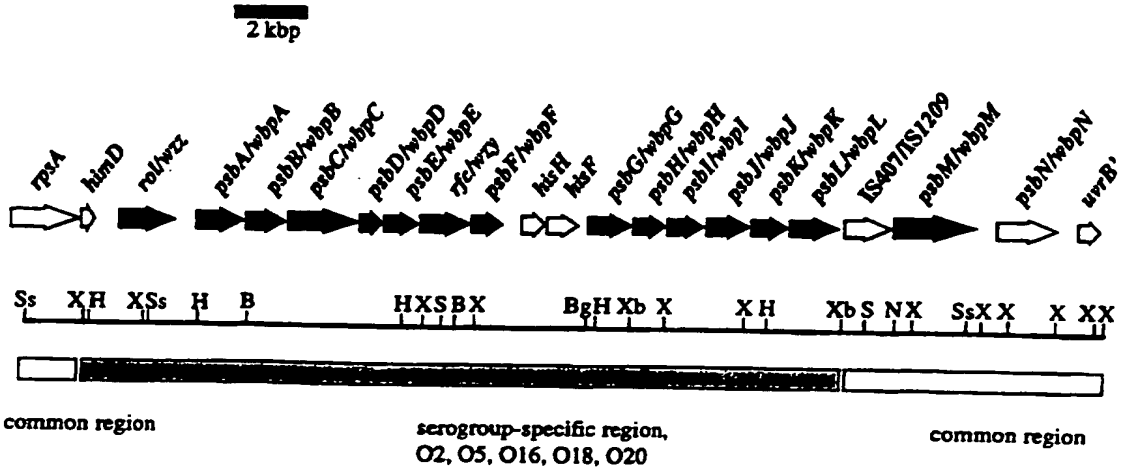
(54) Title: PROTEINS INVOLVED IN THE SYNTHESIS AND ASSEMBLY OF O-ANTIGEN IN *PSEUDOMONAS AERUGINOSA*



The *Pseudomonas aeruginosa* O5 *wbp* gene cluster and flanking DNA

(57) Abstract

Nucleic acid molecules encoding proteins involved in the synthesis and assembly of O-antigen in *P. aeruginosa*; and proteins encoded by the nucleic acid molecules are described. Methods are disclosed for detecting *P. aeruginosa* in a sample by determining the presence of the proteins or a nucleic acid molecule encoding the proteins in the sample.

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BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

INTERNATIONAL SEARCH REPORT

Intl. donal Application No
PCT/CA 97/00295

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/31 C07K14/21 C07K16/12 C12Q1/68 G01N33/569
C12N1/21 //(C12N1/21,C12R1:19,1:385)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	INFECTION AND IMMUNITY, vol. 63, no. 5, May 1995, pages 1674-1680, XP002036228 DASGUPTA T. AND LAM J.S.: "Identification of rfbA, involved in B-band lipopolysaccharide biosynthesis in Pseudomonas aeruginosa serotype 05" cited in the application see page 1676; figure 2 see page 1678; figure 7 see page 1679, left-hand column, line 31-35 --- -/--	1-8,11, 12,14

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
8 August 1997	- 2. 12. 97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Macchia, G

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 97/00295

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GENE, vol. 167, no. 1/2, 29 December 1995, pages 81-86, XP002036229 COYNE M.J. AND GOLDBERG J.B.: "Cloning and characterization of the gene (rfc) encoding O-antigen polymerase of Pseudomonas aeruginosa PAO1" see page 82, left-hand column, line 44 - right-hand column, line 18	1-8,11, 12,14
A	MOLECULAR MICROBIOLOGY, vol. 16, no. 3, 1995, pages 565-574, XP002036537 DE KIEVIT T.R. ET AL.: "Molecular cloning and characterization of the rfc gene of Pseudomonas aeruginosa (serotype O5)" see abstract see page 567; figure 2	1-8,11, 12,14
A	LAM J.S. ET AL.: "MOLECULAR BIOLOGY OF PSEUDOMONADS: Genes involved in the biosynthesis of Pseudomonas aeruginosa lipopolysaccharide (chapter 39)" 1996 , NAKAZAWA T. ET AL. EDS. ASM PRESS , WASHINGTON D.C., USA XP002036539 see page 454; figure 1	1-8,11, 12,14
P,X	MOLECULAR MICROBIOLOGY, vol. 22, no. 3, 1996, pages 481-495, XP002036538 BURROWS L.L. ET AL.: "Molecular characterization of the Pseudomonas aeruginosa serotype O5 (PAO1) B-band lipopolysaccharide gene cluster" see the whole document	1-8,11, 12,14
P,X	JOURNAL OF BACTERIOLOGY, vol. 179, no. 5, March 1997, pages 1482-1489, XP002036231 BURROWS L.L. ET AL.: "Pseudomonas aeruginosa B-band O-antigen chain length is modulated by Wzz (Rol)" cited in the application see page 1482 - page 1483, left-hand column, line 11; figure 1	4,8
A	PROC. NATL. ACAD. SCI. USA, vol. 89, November 1992, pages 10716-10720, XP002037116 GOLDBERG J B ET AL: "CLONING AND SURFACE EXPRESSION OF Pseudomonas aeruginosa O ANTIGEN IN Escherichia coli"	

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 97/00295

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MOLECULAR MICROBIOLOGY, vol. 8, no. 4, 1993, pages 771-782, XP002037117 LIGHTFOOT J AND LAM J S: "CHROMOSOMAL MAPPING, EXPRESSION AND SYNTHESIS OF LIPOPOLYSACCHARIDE IN Pseudomonas aeruginosa: A ROLE FOR GUANOSINE DIPHOSPHOMANNOSE (GDP)-D-MANNOSE" cited in the application ---	
A	MOLECULAR MICROBIOLOGY, vol. 13, no. 3, 1994, pages 427-434, XP002036555 EVANS D.J. ET AL.: "The rfb locus from Pseudomonas aeruginosa strain PA103 promotes the expression of O antigen by both LPS-rough and LPS-smooth isolates from cystic fibrosis patients" -----	

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INTERNATIONAL SEARCH REPORT

Ir. ational application No.
PCT/CA 97/00295

Box I bservations where certain laims were found unsearchable (Continuati n f Item 1 f first heet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. Claims 1-14 (all partially)
2. Claims 1-14 (all partially)
3. Claims 1-14 (all partially)
4. Claims 1-14 (all partially)
5. Claims 1-14 (all partially) Please see attached sheet ,/,

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-14 (all partially)

Remark n Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

6. Claims 1-14 (all partially)
7. Claims 1-14 (all partially)
8. Claims 1-14 (all partially)
9. Claims 1-14 (all partially)
10. Claims 1-14 (all partially)
11. Claims 1-14 (all partially)
12. Claims 1-14 (all partially)
13. Claims 1-14 (all partially)
14. Claims 1, 2, 4, 7 (all partially)
15. Claims 1-14 (all partially)
16. Claims 1-14 (all partially)
17. Claims 1-3, 5, 6, 8-14 (all partially)
18. Claims 2-6, 8-14 (all partially)
19. Claims 2-6, 8-14 (all partially)
20. Claims 2-6, 11, 12, 14 (all partially)

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